

January 10, 1975

Few jobs, but plenty of job security

THE graph on our cover this week encapsulates perhaps the greatest problem confronting British universities at present—that of replacing staff.

For every year since 1960 we looked at the classified advertisement section of *Nature* for the first issue in May and the first issue in November. Any British university post of the level of lecturer or above scored one; temporary posts at the lecturer level (there were very few) scored half. The vertical axis therefore represents, roughly, a measure of the long term openings in science in British universities. The graph could be criticised, no doubt, in all sorts of ways as an inadequate sample but the message is clear. Universities have largely stopped hiring, or 1974 contained a statistical fluctuation, or *Nature* has been abandoned as a medium for advertising university posts (British universities generally advertise in two different places). The last proposition was easy to dispatch: nowhere else is there a boom in university advertisements. Statistical fluctuations were ruled out by looking at a larger sample for 1974. So there just aren't the jobs available.

The peak in the mid-1960s, the other salient feature, arose, of course, from the rapid university expansion at that time; indeed in doing a numerical analysis we frequently had difficulty in deciding how many posts were being offered when a university announced that 'several positions are available'!

The policy that university administrations have adopted in the past year, during which severe financial stringency has dictated a widespread freeze in hirings, has been to treat

individual posts which have fallen vacant on their respective merits, and some have said that a year or two of straitened circumstances might positively do good. This is untrue for two reasons. First, when a system with such a high job security ceases to operate normally it works to the detriment of the bright young man trying to move from contract-based employment to a more stable career. Disruption of his job prospects during the most intellectually fertile period of his life represents a tragic waste of education and talent. Second, just as bad news on employment in the chemical industry a few years ago rapidly had an impact on the image of chemistry in schools, it cannot be long before the failure to fill university posts begins to impress students at all levels with a belief that the academic life is being downgraded in national priorities, and that they should not set their sights in that direction. Even if this were a good thing, it is most undesirable that the control should come from external influences rather than from genuine policy decisions made after rational discussion.

It is unlikely that the brakes will be released from universities in the near future; the government doubtless has many other demands on its money which it perceives as more deserving than the universities. In that case, now is the time for universities to respond to the crisis in an imaginative way. Industry, confronted with a constant budget, would find ways of persuading senior or unproductive staff to leave and it is often possible to do this without any great loss of human dignity. Tenure is both a blessing and a curse to the university system; might not the golden handshake alleviate some of the curse?

Unsuitable for children

PROFESSOR Eric Laithwaite did not, as was widely predicted, quite urge his audience of schoolchildren at the Royal Institution Christmas Lectures (and the several hundred thousand television viewers) to regard Newton's laws as open to some fairly fundamental doubts. He did not say that he had found a way of counteracting gravity. What he did, however, in the course of his six lectures, was in a way more damaging to the education of young scientists; he mixed parlour tricks with complicated machines, he chose to enlist the mystified children on his side in a battle with conventional scientists—'abominable no-men' as he called some of them—and, worst of all, he emphasised experimentation to the almost complete exclusion of explanation.

The scene was as it ever had been—a lecture hall full of well groomed children on their best behaviour. One wondered idly if there were still nannies, governesses and footmen waiting outside in carriages. The audience was prepared to applaud dutifully the most trivial thing. And the experiments—well they can never have been bettered for ingenuity and visual impact.

But science demands rational explanations. It is simply no good demonstrating at length the celebrated mirror problem (if right becomes left, why doesn't top become bottom?) without going to equal pains to put in children's minds at least some sort of answer. It is no good demonstrating a motor that reverses direction when the voltage

is increased without saying why. And it is positively harmful to venture into the world of gyroscopes, a confusing world for many a competent scientist, without a clear understanding of the rather well known laws of angular motion, an extensive reading of the not inconsiderable nineteenth century literature on gyroscopes and tops and an ability to impart the textbook explanation lucidly. The theory is indeed dry and unattractive; all the more challenge, then, to get it right and still hold your audience. Professor Laithwaite chose, instead, to present the understanding of gyroscopic motion as something which had been languishing for more than a hundred years and which scientists as a whole had chosen both to ignore and to suppress questions on.

One example of the misleadingness of demonstration must suffice. A gyroscope slowly precessing on an 'Eiffel tower' seems to experience no centrifugal force, said Laithwaite, otherwise surely the tower would slide, which it didn't do even when the base was on ice, a surface with a very low coefficient of friction. But not low enough. The centrifugal term is sufficiently much smaller than the normal reaction from the weight of the gyroscope (perhaps one hundredth) that sliding requires an even more slippery surface than ice.

These lectures, said Professor Laithwaite, were not the time nor the place to go into the mathematics; but if not, it was neither the time to launch ideas which could only be criticised by mathematical analysis. □



Between combine harvester and ribosome

An Agricultural Research Council/Medical Research Council committee has recently produced a report on food and nutrition research. John Rivers of the Nuffield Institute of Comparative Medicine, London examines its significance.

PERIODICALLY, action by government committees and individuals in this country has resulted in the science of nutrition being rescued from its characteristic doldrums and becoming a national priority. A government inquiry into the physique of recruits for the Boer War got the subject moving in the first place, and later action by people like Lord Boyd-Orr and Sir Jack Drummond gave a new impetus to its growth. These efforts were successful not least because of the military importance of a healthy population. Since 1945, nutrition in the UK has been neither of military importance nor Nobel Prize ranking. It has lost much Research Council support and the standard of the subject and its workers has declined.

The report* of the committee under Professor A. Neuberger is the latest attempt to enumerate nutritional priorities and halt that decline. It examines not only the nutritional status of Britain, but the state of our nutritional knowledge and recommends guidelines for the development of the subject until the end of the century. The strategy adopted is praiseworthy. Priority, it is argued, must be given to "areas of special importance or urgency". Three such are identified—the maintenance and safety of our food supply, the role of nutrition in diseases of complex or uncertain origin, and the extension of our knowledge of the metabolism of nutrients.

Professor Neuberger's committee were asked to prepare a report for both the Agricultural Research Council (ARC) and the Medical Research Council (MRC), an event which is in itself a milestone. The secretaries of the councils welcome it "as a basis for discussion both within the councils and in the wider scientific community preparatory to the consideration of policy". So far the wider scientific community seems, publicly at least, to have been somewhat muted in its discussion. There is therefore the real

possibility that the Neuberger report could, without challenge, become the policy document. This would be a pity, because, despite all its outstanding qualities, the report is a markedly flawed document. The committee has managed to outline graphically some of the priorities of nutrition, but they have remained oblivious of others. The net result is a report which could set back the overall subject by 25 years.

The bulk of the report is a detailed summary of the present state of knowledge in nutrition. Since something over a gross of experts have been consulted, this makes breathtaking reading. If it were expanded and in parts rewritten it would become a necessary primer in nutrition but, as it is now, its function is obscure. It is too condensed to serve as a text book, it is unreferenced and so of little use as a source book, and it is too high powered to be of use as a guide to civil servants working for the research councils or to trustees of foundations. If it is merely there to impress, it succeeds. Moreover it does so in a way that eloquently illustrates Professor Neuberger's conviction that "the answers to many of the most difficult problems in nutrition are ultimately to be found at the molecular or cellular level of biological organisation".

Sir Harold Himsworth—formerly Secretary of the MRC—held a comparable opinion, and some nutritionists felt then that this was a sadly mistaken and reactionary view. Probably they are even sadder now that Professor Neuberger has revived it. Both Himsworth and Neuberger may have described what the MRC choose to fund, but they are not discussing nutrition. In fact they are describing just what nutrition is not. The subject falls somewhere between the combine harvester and the ribosome, but it is not agricultural engineering nor is it molecular biology. It may draw from both but is identical with neither, and it is to be hoped that research councils are aware of its limits when considering which "nutrition projects" to support.

Human nutrition is about the interaction of Man, men and food. It is both a social and a biological science, the domain of generalists who may have some special expertise but remain generalists. Those answers which can only be found at the molecular level, or indeed the economic level, are answers to problems in molecular biology or economics. Both are im-

portant and both should receive funding, but not with money designated for nutrition research.

The apparent erudition of the Neuberger report tempts one, however, to accept the committee's view. The complexity of its science makes it difficult to argue with its insight. Jehovah's Witnesses quote the Bible for much the same effect, but fortunately Professor Neuberger's sources are neither as infallible nor as all-embracing. The result is a review which is eclectic, not exhaustive. Much of what it says is exciting but what it omits is terrifying. Seventy-five pages, for example, are devoted to physiology and biochemistry but only one to ways of influencing food consumption patterns. Even the stress given to different metabolic problems is difficult to understand, unless the length of the contribution is proportional to the status of the author. So although protein and energy metabolism are elegantly viewed from every angle, and coordinated proposals for research in metabolic aspects of obesity advanced, subjects like sucrose and essential fatty acids (EFA) are hardly mentioned.

Yet sucrose provides 15% of the energy of a UK diet—which is a higher fraction than protein supplies. This is a level of consumption which alarms many nutritionists and can delight nobody except the British Sugar Bureau. For some reason the nutrition establishment in the UK seems to regard neither sucrose nor EFA as respectable areas of nutritional research. Perhaps that is the reason why the 27 lines that are devoted to EFA and prostaglandins are so poor. They are marred by misleading omissions, by an erroneous summary of the evidence relating to human requirements and the idiosyncratic phrase "membranes of structural lipids". But the committee's incorrect assertions that "all prostaglandins are metabolically derived from arachidonic acid" or that " γ -lindenic acid is the immediate precursor of arachidonic acid" make the review not only superficial but worthless.

Similar problems occur elsewhere, so that the overall impression is of a report biased in its summary of the literature. Every scientific review is, of course, similarly biased, but since this one gives no references it is impossible to tell whether the author or the reader is ignorant of the literature. Would an unbiased reader really

**Food and Nutrition Research*, (HMSO £3.80).

conclude that studies on high doses of vitamin C have resulted in "very little sound evidence" that it can have any beneficial effect and "some reason to suspect it may be harmful"? Or would he remain open minded?

But the scientific review is only the sprat. The mackerel is the set of policy proposals found, in the main, as terse sentences interspersed with the reviews, and designed to give practical expression to Neuberger's three priorities.

Not one of these can be objected to; they are all worth investigation. But then, given enough time and money, so is Uri Geller. If the proposals are considered as the expression of priorities in nutrition many must be rejected. They are the thoughts of experts enthusing over past triumphs and the importance of what they wish to do next. Such enthusiasm is of importance in science, but its expression in this report is sufficiently uncontrolled that a fusillade of ideas have scattered like buckshot around the target. Although Professor Neuberger has sometimes given due priority to an outstanding research need—for example the whole subject of protein and energy metabolism—he has done a disservice by failing to regard any aspect of metabolism as less than a priority.

The committee's approach to the use of animal models also seems to lack coordination. Such models are lacking for many of the intractable problems of nutrition, and the common laboratory species are perversely disinclined to suffer from obesity, diabetes mellitus, kwashiorkor, ischaemic heart disease and many of the other great nutritional scourges. Which is probably why the diseases remain; if the guinea pig possessed L-gulanolactone oxidase, scurvy might pose quite formidable research problems. Primates are proposed at a number of points as a useful model and the limited usefulness of other animals mentioned.

In their views on primates the committee holds an Aristotelian view of animal relationships as a *scala naturae*. This is a view that has been criticised elsewhere with the admonition to choose the model for its particular functional similarity to man rather than on the basis of some vague evolutionary notion of affinity.

There is a case to be made for giving priority to comparative nutrition if we are ever to understand human nutritional disease. Interspecific generalisations like the relationship between energy expenditure and body weight can only improve our perspective on man. When the similarities are established even the species differences can be valuable. If, as the committee suggest, a considerable proportion of basal metabolic rate is accounted for by the energy cost of protein turnover,

how does the cat have a normal energy requirement but a protein requirement twice the interspecific mean?

But above all a comparative approach might teach us to look at animals, their diets and their diseases as an ecological whole. Nutritional epizootology could do much to counteract the myopia of nutritional epidemiology.



Would it work now?

Myopic, too, is the only way to describe the neglect shown by the Neuberger committee of the whole subject of social nutrition. Precious little value is placed on studies of what people eat—and virtually none on the important field of why they eat it, and why they do not. How do we persuade a person to eat what we regard as good, and avoid what we regard as harmful? No amount of heavyweight basic science in nutrition can avoid the fact that food that is not eaten has no nutritional value. Nor can any amount of enthusiasm about action at the molecular level avoid the responsibility of the nutritionists to improve human diets. That responsibility is all too often not discharged because neither nutritionists or physicians know how to alter effectively what people eat.

The omission of any discussion on social nutrition is a curiously blinkered attitude. If it was an error it will no doubt be as unfortunate as it is inexplicable. What young research worker of 'potential' will be attracted now into social nutrition? The subject, anyway, is discouraging—the experiments are often equivocal and research funds are scarce. Presumably funds will now dry up altogether.

It is a pity that Neuberger did not see fit to review the classic work of Barker, Barnicott, Burnett, Douglas,

Joy and Yudkin, or any of the other authors who seem to be aware that food is something more than the sum of its nutrients. If consulted, their views on priorities would have been something more than a lame note on how "it would be of considerable interest to review the results of advertising campaigns . . . and to assess the effect of these on the nutritional value of the diet". We do not know with any precision why or how the UK diet is changing. We do not know why nutrition education fails when advertising works.

Nutritionists are not in business to pursue uncluttered academic research but to improve the nutritional status of the population. Yet they don't know how except by draconian war-time rationing. Meanwhile the pragmatic, arcane arts of the advertising industry are being neither studied nor tested by the academic world.

In the developing world, our knowledge is even more limited. Although nutrition education has proved ineffective as a method of achieving dietary change in the UK it still flourishes as the panacea for Africa's ills. Only recently has there been any serious attempts to see if it works and why it fails. Even now the analyses are oversimplified—for example, no serious attempts are made to identify socio-economic behaviour. Africans are lumped together, or at best split into tribes and nations.

No less urgent is our need to study the sociology of nutritional science. Some field nutritionists have felt that, despite their best efforts, their role as an alien expert inevitably has an overture of racial arrogance. At its worst this is a caricature: the western bourgeois explaining to an African lady how she should prepare one of her own traditional recipes. But even at its least offensive it remains—western man and his science in judgement on another culture and its beliefs. Western man cannot even claim always to have been correct. The life and death of the protein myth, and the distorting effect it has had not only on aid but on the overall development of nutritional science, needs not merely comment but a detailed critical analysis. If we wish to change nutrition, we must understand its structure.

There are two views about the 'golden age' of nutrition. Many scientists feel that this was the vitamin era before the war, when nutrition and biochemistry were synonymous. But to the public it was of most use during the Second World War, when the state of nutrition of the nation improved. It is with use that one must be concerned, and if practical nutrition were given as high a priority as basic science, its 'golden age' could yet be to come. □

international news

USA ratifies chemical warfare protocol

by Colin Norman, Washington

AFTER nearly fifty years of wrangling, the United States government last month belatedly ratified the most important chemical warfare treaty ever negotiated—the 1925 Geneva Protocol, which outlaws the first use in war of chemical weapons. And, for good measure, final approval was also given to a 1972 treaty which forbids the development, production and stockpiling of biological weapons.

But, encouraging as those actions are, they unfortunately do not signify that the Pentagon has lost interest in chemical weapons, nor do they indicate that the Administration is yet prepared to negotiate seriously for international chemical disarmament. Any lingering doubts on that score were dispelled late in December when it became known that even as the Senate was about to approve the Geneva Protocol, the Army was blithely pushing ahead with its plans to produce a new generation of nerve gas weapons called binaries.

For years, the Army Chemical Corps has been conducting research and development on binaries with a view to replacing its ageing stockpiles of nerve agents—estimated to amount to about 40 million pounds—with the new weapons. The idea is that since binaries consists of two relatively non-toxic components which form a lethal agent only when they are mixed together, they can be manufactured and stored much more safely than conventional nerve gases.

But last year Congress refused to grant any money for the production of binary weapons, chiefly because of arguments that if the United States were to embark on a massive new chemical weapons programme, any chances of negotiating an international chemical disarmament (as opposed to no-first-use) treaty would almost certainly have been wrecked. Such negotiations have been taking place in Geneva for the past five years under the auspices of the Conference of the Committee on Disarmament (CCD); it would clearly be difficult for the United States representatives at the talks to retain any credibility if they were placed in the position of arguing for chemical restraint while the Army is busily building up its stockpiles.

Nevertheless, in spite of production ban, the Army published a solicitation in the *Commerce Business Daily* on

December 11, enquiring whether any company would be interested in manufacturing "ton quantities" of two chemical components of binary weapons and also "artillery projectiles filled with a non-toxic chemical solution". That move clearly indicates that the Army has not dropped its plans for binary weapons, and that it will almost certainly be coming back to Congress this year with another request for production funds.

Another aspect of the solicitation is also very interesting. Last year, the Army was planning to produce binary weapons with two chemicals which would form the nerve gas GB when mixed together. But the announcement on December 11 indicates that the Army is now also planning to produce a binary weapon which would form the much more persistent nerve agent VX. The gas GB quickly breaks down in the environment, but VX can remain lethal for several weeks under some conditions.

So the Army is clearly hoping to push ahead with its chemical weapons programme, but it will have a tough domestic fight on its hands. For one thing, the arguments which persuaded Congress to shut off production funds last year will be just as strong this year, added to which the new Congress is expected to be politically more liberal than the last. And for another, the

THE announcement last month that the Ford Administration has come round to the view that the Geneva Protocol includes herbicides and riot control agents places the British government in an isolated position. In February 1970 Mr Michael Stewart, then Foreign Secretary, announced in the House of Commons that the British government considers that "CS and other such gases" are outside the scope of the protocol. Although the protocol clearly says nothing about non-military use of chemical weapons, the reason for Mr Stewart's decision was believed to be the fact that CS was being used extensively in Ulster. Even at the time, such an interpretation was shared by few other countries.

● THE British Government was further embarrassed over its policy on nerve gases this week when a *Sunday Times* report revealed that the formula for the lethal VX gas had been

taken off the secret list, and was available to anyone who cared to spend an afternoon browsing in the Patent Office.

The Minister of State for Defence, Mr. Rodgers has agreed to examine the procedures followed when a chemical weapon is taken off the the secret list. He gave the undertaking in a letter to Mr. Bruce Douglas-Mann, a Labour MP who tabled two questions to the Defence Secretary Mr. Mason, and expressed fears that the V-agent would be a dangerous weapon if it fell into the hands of terrorists.

Anybody can now obtain a detailed account of the process by which VX is made by applying to the Patent Office, where details were filed 14 years ago as a classified specification. Part of the patent papers were actually reproduced in the *Sunday Times* in order to indicate their availability, and the report suggested that a student, with a little ingenuity, could manage to manufacture the gas in a university labora-

tory. A photograph of the manufacturing apparatus, published after an 'open day' at the chemical warfare plant at Nancekuke, Cornwall, makes the job of the terrorist chemist that much easier.

The nerve gas codenamed VX is the most toxic of a family of V-agents produced by British chemical warfare scientists since World War Two. The gases are organic-phosphorous compounds, and were synthesised in the mid-1950s by a team at the Chemical Defence Experimental Establishment at Porton Down, Wiltshire. V-agents were not produced in bulk in the UK, but under an information-exchange agreement between Britain and America, details of the process were passed on, and formed the basis of a weapons system developed by the US Department of Defence. VX is said to be so toxic that in liquid form a drop the size of a pinhead, placed on the skin, is lethal.

Administration has been carrying out its own internal review of the binary programme. The matter is now said to be awaiting the attention of Secretary of State Henry Kissinger, and it is no secret that the Arms Control and Disarmament Agency (ACDA) has been vigorously opposing production of the weapons. It would clearly be in the Administration's political interest to abort the programme itself rather than face another hopeless battle with Congress.

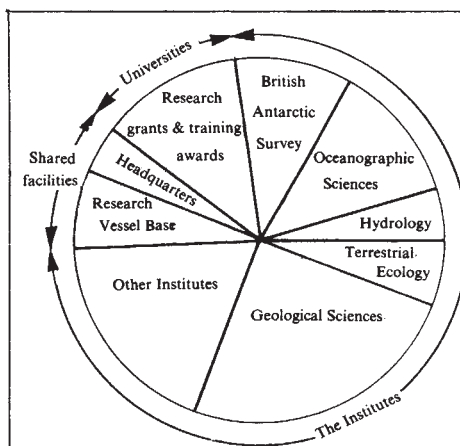
Moreover, even in military terms, the Pentagon may come to realise that the programme just is not worth the candle. It has been estimated that it will cost at least \$1,000 million to replace existing nerve gas stockpiles with binaries and since the Pentagon is always complaining about the inadequacy of the defence budget, it should be asking itself whether the money could better be applied elsewhere.

But, the ratification last month of the Geneva Protocol may at least help the CCD talks when they resume in Geneva this spring. The fact that the United States had not even ratified the 50-year-old treaty barring first use of chemical weapons was viewed by several delegations in Geneva—particularly the Soviet delegation—as evidence that the United States was not really interested in chemical disarmament, but at least that stumbling block has now been removed.

The problems with the protocol came in two parts when it was originally submitted to the Senate for ratification. (All such treaties require a two-thirds majority vote in the Senate before they become official US policy.) First, it fell foul of the chemical industry and was never brought to a vote; after languishing in the Senate Foreign Relations Committee for years, it was eventually withdrawn from the Senate. Then, in 1971, President Nixon resubmitted the treaty to the Senate for ratification.

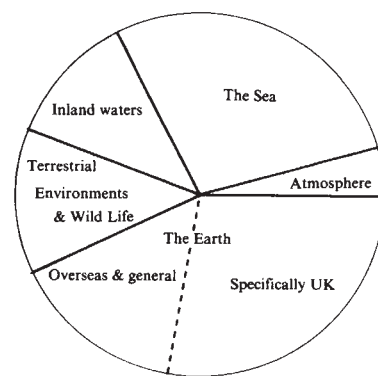
But another impasse developed because Nixon insisted that herbicides and riot control agents are not covered by the protocol an interpretation which would have meant that, even if the United States ratified the treaty, it could continue using herbicides and tear gases in Vietnam. Senator J. W. Fulbright, Chairman of the Senate Foreign Relations Committee, did not, however, accept that interpretation. He refused to bring the measure to a vote and asked the Administration to review its position.

There the matter rested for three and a half years until Fulbright called a meeting of the Foreign Relations Committee on December 10 last year to review the situation. Faced with another embarrassing blaze of publicity the Administration finally overruled the



THE British Natural Environment Research Council (NERC) saw through half of the total Rothschild transfers in the year 1973-74 according to its recently published report (HMSO, £1.50). For that year £3.6 million worth of research was assigned to customer departments, for the most part satisfactorily—meaning that everyone carries on much as before including the NERC which goes on managing. The NERC does, however, sound a warning signal. The council doesn't reckon it has enough applied research under its wing to meet the Rothschild targets, so some "quite basic research" is being transferred to departments. The question is whether in lean times ahead basic research in a customer department's budget will present a "conflict of priorities", a delicate term for being axed.

Some of the transferred programmes do not sit too easily in customer departments: the NERC gives two examples. The geological survey of the continental shelf is a hard-to-place baby. There is £1.5 million of applied research to be transferred, but the first home for it, the Chemical and Minerals Board of the Department of Trade and Industry, turned out to be less than ideal. It looks as if it can find a foster-home with the Shipping and Marine Technology Board of the



NERC's £19 million cake divided—by means and by ends.

same department but the NERC would like to see it finally adopted by the Department of Energy. On the other hand, the land Geological Survey is a much sought-after property and in order to prevent it from being torn limb from limb, the NERC has agreed to the survey being overseen by a consortium comprising, *inter alia*, the NERC's chairman, Sir Peter Kent, and representatives of the departments of Trade, Industry, Environment, Scottish Development and of the Welsh office.

The Institute of Geological Sciences is reported to be finding difficulty in recruiting and keeping staff to work on the independent analysis of North Sea commercial data that it attempts to carry out. Uncompetitive salaries in the scientific civil service are blamed.

Further, significantly fewer people applied for research studentships this year, the report notes, but those that did were distinctly more willing to move to a different university for their second degree.

As the figure shows, universities continue to comprise only a modest fraction of the NERC's commitment. Britain's terrestrial toe-hold in the Antarctic, gets almost as much as the total expenditure in universities.

Pentagon, and Dr Fred Iklé, Director of ACDA, announced that President Ford now accepts that the protocol bars first use in war of all chemical weapons, including herbicides and tear gases. The measure then sailed through the Senate without opposition.

The actual effect of all this on progress in the CCD talks is uncertain at present, largely because of the looming threat of the binary programme. But, assuming that talk of producing binaries dies away this year, the chief stumbling block in Geneva will become the difficulty of making sure that a chemical disarmament treaty can not

be violated. One possibility, however, is that a treaty banning further production of lethal chemical weapons will first be negotiated. That would prevent chemical proliferation—and also reduce the capability of both the United States and the Soviet Union, since existing nerve gas stocks will slowly deteriorate. A ban on stockpiling would then be negotiated later. A Japanese proposal along those lines is now receiving close attention in the United States.

The key to the whole business, however, is what happens to the binary programme. □

WHILE international attention has been focused on plans for nuclear power stations in Israel (and neighbouring countries), Israeli scientists have continued their search for new sources of energy, or for more efficient means of exploiting old sources. Everything is being considered, from the proposal of a scientist at the Haifa Technion to construct 10,000 giant energy-generating windmills to the scheme of his colleague for a thermonuclear power reactor.

Research continues on an obvious energy source in this sub-tropical climate—the Sun. Unfortunately, solar energy has not proved itself economically viable except for the heating of household water supplies. And its benefits in this sphere are cancelled out, in the eyes of environmentalists, by the ugliness of the roof-top solar energy installations which, together with the omnipresent television aerials, manage to create a particularly horrifying urban skyline.

Even though Israel has far more ample supplies of solar radiation than of water, there is even talk of constructing a 120-MW hydroelectric station near the northern end of the Sea of Galilee.

A small hydroelectric station was actually opened on the River Jordan, south of the Sea of Galilee, way back in 1932, but it has been out of action since the 1948 war. The proposed new plant would be placed at a point where

the Jordan plunges 830 feet down into the northern end of the Sea of Galilee.

Utilisation of oil shale is also under active consideration thanks to Professor Yehoshua Schachter of Bar-Ilan University, an Orthodox Jewish institution where all the scientists wear skullcaps. Professor Schachter believes that the estimated 700 million tons of oil shale recently discovered in the Negev Desert would be sufficient to generate some

Letter from Israel

from Nechemia Meyers, Tel Aviv

1,000 MW of electricity for 30 years. This is an impressive figure in Israeli terms, as the country's present generating capacity is 1,600 MW.

Schachter's plan, now being studied by a government-appointed engineering company, calls for the shale to be burnt on-site to generate electricity, rather than for the oil to be extracted first and then burnt at a power station. Such a direct burning process has been used successfully in West Germany and Estonia, and skyrocketing fuel prices have made it look attractive here.

Professor Schachter also emphasises the fact that the power-generating facility he suggests would be particularly valuable in wartime, when other fuel imports might be cut off.

● War, of course, could come here at almost any moment, and in the mean-

time Israel has a full-scale anti-terror campaign of her hands, one in which scientists have already played some role. Their contributions, unfortunately, have not always been utilised. For example, a reliable Israeli-developed device for 'sniffing' explosives was not used to check the explosives-laden suitcase of a young man who arrived recently at Lydda (Ben-Gurion) Airport. The next day he used the explosives to make bombs which he subsequently exploded inside a Tel Aviv cinema.

● Israel's special problems made it the natural venue for an International Conference on Psychological Stress and Adjustment in Time of War and Peace, which opened at the University of Tel Aviv on January 6. One of the papers presented at the conference described a study carried out by Dr. Avner Ziv, University of Tel Aviv, on 'shelter children', youngsters in border areas who must spend extended periods in underground shelters because of recurrent shellings. Dr Ziv and his colleagues found that kibbutz children under bombardment adjusted to the stresses of the situation much more successfully than did children at nearby small towns. He speculates that the highly organised social structure of a kibbutz, and the fact that it operates almost like an extended family, may provide a more secure atmosphere for children in times of crisis than does the nuclear family in a town.

AFTER a year and a half of actively, but silently consulting everybody, the Dutch Science Minister Mr F. H. P. Trip, finally ventured into the limelight to present his 'Purple Book' on science policy.

Mr Trip is a most remarkable man. He has roots in industry (as a managing director of Naarden Chemie, a specialised company with more people in its laboratories than in its production departments) and in academe (as President-elect of Utrecht State University), so many regarded him as the ideal science manager. Also, surprisingly, he is a member of one of the most radical political parties in the Netherlands.

Yet, according to his new plans, he is not after radical changes in the structure of science policy. The universities maintain their rate of income per student and their freedom to handle the sum of Dfl. 1,000 million as they please. But they are requested, if not required, to set up a reasonable book-keeping system for their research projects, in accordance with the government's technical guidelines. In view of the present state of the average university administration, implementation of this rule will call for a major effort.

Mr Trip's purple book

from Arie de Kool, Rotterdam

Something like Dfl. 100 million a year is now handed out by the ZWO, the organisation for pure scientific research. It is rightly being claimed that this rather insignificant amount has a very considerable steering effect. The Foundation for Basic Medical Research funded by the ZWO, claims to coordinate about Dfl. 80 million worth of research on a budget of Dfl. 4 million. They get there by adding a man here and a centrifuge there—on conditions, of course. The ZWO (and the Royal Academy, as far as its own institutes are concerned) will be transformed into a Council for Scientific Research (RWO), with departments for fields like physics, life sciences and social sciences. Each department could have many 'working groups', consisting basically of everybody working in a specific discipline. There could be working groups for solid state physics, molecular biology, sociology of the family and so on.

The working groups elect ten mem-

bers of the department; five others are appointed by the Academic Council (the cooperative institution of the universities), and five more, together with a president, by the government.

Some Dfl. 1,000 million is spent on more or less applied research sponsored by the government. This will stay under direct ministerial control although the ministers concerned will receive advice, and even planning schemes to cover several years from sectorial councils, such as a council for environmental problems, one for maritime research or the environmental movement.

The councils are to be meeting points for government and producers and consumers of science. So each council consists of some government representatives, some scientists from the institutes concerned and some people from 'society'—industrialists, labour union representatives and people from the environmental movement.

Mr Trip also hopes that industry (in the Netherlands some 75% of industrial research and development is done by five multinational companies) will be open about its own projects, so that good coordination of government-funded research and development will be possible. □

correspondence

UNESCO

SIR,—As visiting scientists at the Weizmann Institute, we find the recent UNESCO anti-Israel resolution doubly abhorrent.

First, as scientists and educators, we are appalled at the biased, politicised and cowardly vote, which has made a travesty of an organisation established to help wipe out just such ignorance and prejudice.

Second, being in Israel we have had the opportunity to explore Jerusalem—the old, the new, the restored, the excavated—and thus to recognise the absurdity of the claim that the excavations carried out in the Holy City have changed its character.

We call on our fellow scientists to join us in protesting UNESCO's moral bankruptcy and in demanding that the organisation abandon politics and return to dealing with science, education and culture.

Yours faithfully,

L. ANDERSON (University of Illinois), E. E. A. BROMBERG (University of Wisconsin), C. BRUNK (University of California), D. CAHEN (Northwestern University), A. COGOLI (ETH, Zurich), S. COHEN (State University College at Buffalo), R. COOPER (University of Pennsylvania), B. S. DUDOCK (State University of New York at Stony Brook), S. EDELSTEIN (Cornell University), D. FAIMAN (CERN), A. FRIMER (Harvard University), R. A. GELMAN (Case Western Reserve University), A. KRSTOSEK (University of Oregon), S. J. LEIBOVICH (University of Washington), J. MANZ (Technische Universität, Munich), S. B. MIZEL (Colorado State University), J. PRIVES (Columbia University), H. SCHMITT (Free University of Brussels), J. SCHULTZ (University of California), P. B. SIGLER (University of Chicago), A. SOUDAK (University of British Columbia), M. THALER (University of California), S. WEISROSE (University of London), H. YAMASAKI (Hiroshima University), M. ZEICHER (Vrije Universiteit, Belgium).

Another African Chalicothere

SIR,—Among a large collection of vertebrate fossils from the Lukeino Formation which I made recently I found the proximal phalanx of a large Chalicothere. Its size suggests that it belongs to *Ancylotherium hennigi* Dietrich, although if so that species will prove to be the oldest known specimen (~6.5 Myr).

Chalicotheres are now known from several deposits in Africa—the Lower Miocene of Rusinga and Songhor, Kenya; the Pliocene of Kaiso, Uganda, from which the first African Chalicothere was recognised (C. W. Andrews, *Nature*, **112**, 696; 1923); and Lower Pleistocene deposits such as Olduvai, Tanzania (P. M. Butler, *Bull. Br. Mus. Nat. Hist. Geol.*, **10**, 165–237; 1965). In the Baringo area Kenya, they have been recognised from the Chemeron Formation (~4 Myr.) and now from the Lekeino Formation.



On discovery of the Lukeino fossil, I described a Chalicothere to my field assistant, Mr Kiptalam Chepboi, who assured me that I had accurately described a *Chemosit*. I repeated the description to several other local people, all of whom gave the same opinion. The *Chemosit* is an animal of Kalenjin myth, on which the 'Nandi Bear' is supposed to have been based (*Nature*, **112**, 696; 1923 and B. Heuvelmans, *On the Track of Unknown Animals*, Rupert Hart-Davis, London; 1958) and it was the first discovery of a Chalicothere in Africa that prompted Andrews to enquire whether the Chalicothere was still extant and whether it formed the basis of the myth. It is therefore of great interest to obtain further information from an independent source, separated by more than two generations and hundreds of miles, that indeed the *Chemosit* and the Chalicothere closely resemble one another.

It is unlikely that the Chalicothere is still extant, even in the depths of the Zaire forests, but it is not inconceivable that it survived until the recent past, entering local mythology before finally dying out.

MARTIN PICKFORD
Queen Mary College, London

Asbestosis

SIR,—W. P. Howard's comment (December 13) on Peter J. Smith's article (October 18), "For those in peril on the factory floor" which dealt with the industrial hazards of asbestos, itself deserves comment. It is true that conditions in the industry are incomparably better than they were in some factories 40 years ago but the occurrence of an average of 139 new cases of asbestosis a year in Britain still emphasises a formidable hazard. Mr Howard, writing from a London office where he only inhales the few asbestos fibres normally present in an urban atmosphere, may have every confidence that in coming years the number of cases will be reduced to a very low level indeed, but this confidence suggests a complacency that is not, I am sure, characteristic of the industry as a whole. We do not know to what level the concentration of asbestos in the atmosphere must be reduced before the hazard will disappear. The Maximum Admissible Concentration level is a guess.

That asbestos is a dangerous commodity cannot be too strongly emphasised and, although it is indispensable for certain purposes, it should be used with discretion. Because of television programmes describing the occurrence of asbestosis and cancer in workers in Hebden Bridge, the public has become aware of dangers that can arise when the material is handled indiscriminately. A general review (*The Biological Effects of Asbestos*, distributed by the World Health Organisation) that summarises the report of a working conference on the biological effects of asbestos, sponsored by the International Agency for Research on Cancer, indicates that all the major commercial types of asbestos can cause cancer and that there is strong evidence to relate past exposure to asbestos with mesotheliomas, a distressing cancer of the pleura that covers the lung.

The danger may not relate only to employees. One paper in the same report represents a study of the incidence of mesotheliomas in the city of Hamburg-Bergedorf in the years 1958–68. In the city as a whole the incidence was 0.056%; in the residential area around one factory it was 0.96%. Cases considered to be occupational were excluded.

P. F. HOLT
University of Reading

Research in India

It is only natural that Dr Macdonald (*Nature*, November 15, 1974)—with his World Health Organisation (WHO) and Genetic Control of Mosquito Unit (GCMU) connections—should have reacted the way he has, to a part of my item on work on the genetic control of mosquitoes (*Nature*, September 20, 1974). I am afraid I cannot agree that there was anything “misleading”, “misinformed” or “erroneous” in statements which pointed out disparities between population needs and the GCMU’s priorities in choosing its programmes. The military implications too cannot be summarily dismissed, as Dr Macdonald has done, by merely hiding behind the familiar generalisation that all research results, in theory, could be misused. The fact of the matter is that there have been instances of misapplications. It may well be recalled that the defoliants employed by the US Army in Vietnam were reported to have been first tested for their effects in some Latin American countries. I cannot say what were the specific long-term benefits from these tests to the Latin American Communities.

Then there is the case of childhood malnutrition. For years now, we have been led to believe (by all kinds of agencies including UN and others) that protein deficiency in the Indian diet is the major cause of malnutrition in India. Consequently, vast amounts of Indian (as well as foreign) money were diverted towards high-protein food programmes, whereas the problem all along—we are now told—has been one of simply not enough food.

Moreover, studies “specifically designed for the long-term benefit of the community” should surely be able to stand a little scrutiny, and a probe into the whole affair can only result in clearing the air and, I hope, lend support to contentions like Dr Macdonald’s, and I and many others in the scientific community shall be happier for it. Little inconveniences like these, I am afraid, will remain a foreign scientist’s lot (not only in India but elsewhere) so long as instances of the academic and scientific communities providing cover for nefarious activities by their governments continue to surface.

Yours faithfully,

NARENDER K. SEHGAL

Jullundur

SIR,—The belated comments on United States Defense Department and National Museum activities in India by N. K. Sehgal and A. N. D. Nanavati (*Nature*, September 20, November 29) need to be considered in relation to

some other past history.

So far as I can make out the most revealing information is provided by William E. Small in *Scientific Research* (3, (25), 27; December 9, 1968), where investigations of bird diseases transmissible to man in Brazil and the biology of the north central Pacific by the Smithsonian Institution (the US National Museum) were identified as supported by the US Defense Department chemical and biological (CB) warfare research centre at Fort Detrick in Maryland. The resulting furore reached a climax the following February, as reported in the *Washington Post* and *New York Times* on February 5 and the *London Times* and *Guardian* next day, where it is alleged that Baker Island in the central Pacific had been chosen for tests. Eventually President Nixon announced on November 25, 1969 that the USA would abandon CB warfare except for a small defensive programme, though the *Times* reported on September 21, 1971 that stocks of tularaemia, anthrax, Q fever and Venezuela equine encephalitis organisms were retained and work continued at Dugway, Utah, the place where 6,000 sheep were inadvertently killed in a mismanaged nerve gas experiment in March 1968.

Although this activity may suggest that the US Defense Department, in collaboration with the Smithsonian Institution, also financed a Migratory Animal Pathological Survey from Korea through the Far East and India to the eastern Mediterranean at much the same time, as far as one can make out the actual work was normally delegated to irreproachably upright citizens who succeeded in making very good use of the funds provided, to such an extent that the people most likely to take offence, the Russians (who were incidentally carrying out similar activities of their own), were happy to co-operate, as reported by Mr Nanavati, though the Chinese refused to do so. In consequence the withdrawal of US funds following criticism of the Defense Department has resulted in a sad gap in ornithological work in the Far East especially, where one could wish for a less controversial alternative source of money. Mr Sehgal may rest assured that whatever the original object of the Migratory Animal Pathological Survey, a lot of people have scrutinised its activities rather carefully and found nothing to complain about except some roughness in handling captive birds, so that many of us who were once among the foremost critics of its possible original object would now like to see some means found to keep it in being.

As many people have already remarked, it also seems a pity that the

Smithsonian Institution ever allowed its activities to become so closely associated with those of the US Department of Defense, and it hardly seems surprising that people in India remain suspicious.

Yours faithfully,

W. R. P. BOURNE

University of Aberdeen

Ghost families

SIR,—There are even families of ghost writers (December 6). *Proc 5th int. Conf. Soil Mech.* (1961) lists in its Author Index not only F. Asce (II, 105) but also his more prolific younger brother M. Asce (I, 517 and II, 117). Is there a case for forming a Society of Irreproducible Scientific Authors?

Yours faithfully,

PHILIP I. LEWIN

Building Research Station,
Garston, Watford, UK

SIR,—May I add to the Christmas season of ‘ghost authors’ with my own favourite. A paper on a mermaid foetus is quoted in the *Cumulative Index Medicus*, and also by at least one subsequent author, as Williams, H. I., and Lumpur, K., (1962) *Arch. Path. (Chicago)*, 74, 472. In fact reference to the paper itself shows that Dr Williams, the sole author, wrote his report from Kuala Lumpur.

Yours faithfully,

MARTIN D’A. CRAWFURD

The University,
Leeds, UK



A hundred years ago

THE great solar eclipse of 1868 was visible in Siam, as the 1875 eclipse will be. The then reigning Siamese king had not invited any European astronomer; but the French Government sent an expedition, who located themselves in Malacca for the purpose of taking spectroscopic observations. The King of Siam, who professed to be an astronomer, came with a royal train and a large army to observe the sun and perhaps the sun-observers. The observations were very successful indeed; but the French astronomers had located themselves on marshy land and were almost all attacked by fever, of which they were cured only on their return to France. Such was not the case, however, with their royal guest, who was also attacked, and died a few months afterwards.

from *Nature*, 11, 216, January 14, 1875

news and views

Understanding the control of vitamin D synthesis or taking a short cut through South America

from a Correspondent

CHOLECALCIFEROL is classified as a vitamin only because its synthesis from 7-dehydrocholesterol is effected by ultraviolet light rather than by an enzyme. Consequently if there is limited exposure to sunlight, the diet must be supplemented with cholecalciferol. 1,25-dihydroxycholecalciferol ($1,25-(\text{OH})_2\text{D}_3$), the active form of cholecalciferol, is more properly defined as a hormone since it is synthesised solely in the kidney¹ from its immediate precursor 25-hydroxycholecalciferol ($25-(\text{OH})\text{D}_3$). This metabolite is involved in translocation of calcium across cell membranes and in calcium homeostasis, and has potential uses in the treatment of metabolic bone disease. There is therefore much interest in the control of $1,25-(\text{OH})_2\text{D}_3$ production and, since the chemical synthesis of $1,25-(\text{OH})_2\text{D}_3$ cannot be carried out in high yield, in natural sources of the hormone.

The control of $1,25-(\text{OH})_2\text{D}_3$ production has now become a source of controversy with two possible mechanisms being proposed; a recent paper² has indicated the complexity of the relationship of $1,25-(\text{OH})_2\text{D}_3$ with parathyroid hormone. Most groups believe that $1,25-(\text{OH})_2\text{D}_3$ production is primarily under the control of parathyroid hormone. This is supported by the changes which occur in chick mitochondrial $25-(\text{OH})\text{D}_3$ -1-hydroxylase activity in response to changes in parathyroid hormone concentrations³. Also within hours of thyroparathyroidectomy the concentrations of $1,25-(\text{OH})_2\text{D}_3$ fall in blood and intestinal mucosa of rats on a low calcium diet, but injection of parathyroid hormone restores $1,25-(\text{OH})_2\text{D}_3$ concentrations to normal⁴. The rate of conversion of $25-(\text{OH})\text{D}_3$ to $1,25-(\text{OH})_2\text{D}_3$ in an isolated renal tubule preparation can be increased by the addition of parathyroid hormone or cyclic AMP to the incubation media⁵. Both the concentrations of $1,25-(\text{OH})_2\text{D}_3$ in tissues of rats and chicks and the activity of the $25-(\text{OH})\text{D}_3$ -1-hydroxylase enzyme measured *in vitro* are responsive to changes in levels of calcium in the plasma⁴ and media³ respectively. But these groups felt that calcium does not

control $1,25-(\text{OH})_2\text{D}_3$ production: first, because the enzyme activity measured *in vitro* was not related to the plasma calcium concentrations in the bird from which the kidneys were obtained³; second, the transfer of rats from a low calcium to a high calcium diet results in a rapid decline in plasma calcium concentrations whereas the $25-(\text{OH})\text{D}_3$ -1-hydroxylase activity remains elevated for very much longer⁶. Calcium affects the activity of the $25-(\text{OH})\text{D}_3$ -1-hydroxylase enzyme by preventing electrons from flowing into the 1-hydroxylase system from a specific NADPH generating system. Under these conditions added NADPH provides the necessary reducing equivalents⁷.

The alternative mechanism for control of $1,25-(\text{OH})_2\text{D}_3$ production has been proposed by Professor MacIntyre and his group⁸. They have not seen an increased production *in vivo* of $1,25-(\text{OH})_2\text{D}_3$ in response to parathyroid hormone⁹ nor in their isolated renal tubule preparation could parathyroid hormone raise $1,25-(\text{OH})_2\text{D}_3$ production though dibutyryl cyclic AMP did have a positive effect¹⁰. If calcium was omitted from the incubation medium however, parathyroid hormone did stimulate $1,25-(\text{OH})_2\text{D}_3$ production. Since they found added parathyroid hormone to be increasing cyclic AMP concentrations in their isolated renal tubules they have postulated that the influx of calcium into kidney cells, known to occur in response to parathyroid hormone, has negated the effect of the increased cyclic AMP concentrations. They have now incorporated their findings in a hypothesis which proposes that $1,25-(\text{OH})_2\text{D}_3$ production is subject to feedback control.

The key observation was the decreased capacity of renal tubules to synthesise $1,25-(\text{OH})_2\text{D}_3$ if the tubules had first been pre-incubated for 150 min with the hormone. This inhibitory effect of the $1,25-(\text{OH})_2\text{D}_3$ was not due to competition by the hormone for the active sites on the $25-(\text{OH})\text{D}_3$ -1-hydroxylase enzyme and was not detected if the renal tubules were incubated with the hormone in the presence of

actinomycin D. The authors propose that $1,25-(\text{OH})_2\text{D}_3$ stimulates by a nuclear action the synthesis of a protein, possibly calcium-binding protein, which is involved in the mechanism of calcium uptake by kidney cells. In the presence of this protein, tubular calcium is reabsorbed and the consequent raising of kidney cell calcium concentration inhibits $1,25-(\text{OH})_2\text{D}_3$ production. There is therefore a cycle of events in which $1,25-(\text{OH})_2\text{D}_3$ production is controlled by its own action on the tubular reabsorption of calcium.

Crucial to the validity of this hypothesis are the limits to any variation in the concentration of cellular calcium. This information is unfortunately difficult to obtain with confidence because under certain conditions mitochondria take up extremely large amounts of calcium. Even so the changes in $25-(\text{OH})\text{D}_3$ -1-hydroxylase activity which have been observed in response to changes in calcium concentration are inadequate to explain the changes in $1,25-(\text{OH})_2\text{D}_3$ production *in vivo* unless the cell calcium concentration varies over enormous ranges. Thus a 7.5 fold change in calcium concentration gives rise to only a 50% inhibition in $1,25-(\text{OH})_2\text{D}_3$ production *in vitro*, whereas several-fold variations in $1,25-(\text{OH})_2\text{D}_3$ production rates can be observed *in vivo*³.

These variations might, however, have other causes. For example, any measures that lower serum inorganic phosphorous and hence kidney phosphorous concentrations increase $1,25-(\text{OH})_2\text{D}_3$ production⁴. In addition there may be multiple effects of parathyroid hormone on $1,25-(\text{OH})_2\text{D}_3$ synthesis. Favus, Walling and Kimberg² have found that the response of rats to parathyroidectomy varies with time. They found that there is a diminished intestinal calcium transport 72 h after surgery, but parathyroidectomised rats adapted to a low calcium intake over 21 d. This adaptation to a low calcium diet is known to be $1,25-(\text{OH})_2\text{D}_3$ dependent. In interpreting these and other results it should, however, be appreciated that an absolute dependence of $1,25-(\text{OH})_2\text{D}_3$ production on

the presence of parathyroid hormone has never been shown. In parathyroidectomised animals some 25-(OH) $_2$ D $_3$ -1-hydroxylase activity always remains. In accord with this Favus *et al.* found plasma concentrations of the hormone were low but levels in the intestine were normal 21 days after parathyroidectomy.

Recently the South American plant *Solanum malacoxylon* has been seen as a possible source of 1,25-(OH) $_2$ D $_3$. Cattle and other animals eating this shrub show features similar to those of hypervitaminosis D including hypercalcaemia, hyperphosphataemia, vascular and ectopic calcification, wasting and eventual death. The derangement of calcium metabolism probably derives mainly from the increased intestinal absorption of calcium. There is some evidence that the active principle in *S. malacoxylon* is 1,25-(OH) $_2$ D $_3$ rather than the other vitamin D metabolites¹¹.

S. malacoxylon given orally to chicks induces synthesis of calcium-binding protein (CaBP)^{11,12}. This protein seems to be involved in calcium absorption and its synthesis is dependent upon 1,25-(OH) $_2$ D $_3$. The action of the leaf is not inhibited by the inclusion of strontium in the diet, a metal which inhibits the conversion of 25-(OH)D $_3$ to 1,25-(OH) $_2$ D $_3$ by the kidney enzyme system, suggesting that the active principle is not metabolised by the kidney 25-(OH)D $_3$ -1-hydroxylase prior to its action in the intestine. Corradino and Wasserman¹³ have shown that the aqueous extract of the leaf has two other actions similar to that of

1,25-(OH) $_2$ D $_3$. Thus it is active in stimulating within 12 h CaBP synthesis and calcium absorption in organ cultured chick duodenum; again implying that if any metabolism of the active principle is involved it can occur in the intestine. As in the case of 1,25-(OH) $_2$ D $_3$, the induction of CaBP in this system is inhibited by actinomycin D. In addition 1,25-(OH) $_2$ D $_3$ and the aqueous extract of the plant induce *de novo* CaBP synthesis in 18-day-old chick embryo duodenum, whereas 25-(OH)D $_3$ is much less potent in the embryo in this regard and vitamin D seems to be without activity.

Little information is available on the chemical nature of the active principle. If it is 1,25-(OH) $_2$ D $_3$ then its water solubility implies that it is attached to large polar moieties and the hydrolysis of this complex may account for its delayed action^{12,13}. Its identification is awaited with interest.

- ¹ Fraser, D. R., and Kodicek, E., *Nature*, **228**, 764-766 (1970).
- ² Favus, M. J., Walling, M. W., and Kimberg, D. V., *J. clin. Invest.*, **53**, 1139-1148 (1974).
- ³ Fraser, D. R., and Kodicek, E., *Nature new Biol.*, **241**, 163-166 (1973).
- ⁴ DeLuca, H. F., in *Metabolism and Function of Vitamin D* (edit. by Fraser, D. R.), 5 (Biochemical Society, London, 1974).
- ⁵ Rasmussen, H., Wong, M., Bikle, D., and Goodman, D. B. P., *J. clin. Invest.*, **51**, 2502-2504, (1972).
- ⁶ Omdahl, J. L., and DeLuca, H. F., *Physiol. Rev.*, **53**, 327-372 (1973).
- ⁷ Ghazarian, J. G., and DeLuca, H. F., *Archs Biochem. Biophys.*, **160**, 63-72 (1974).
- ⁸ Larkins, R. G., MacAuley, S. J., and MacIntyre, I., *Nature*, **252**, 412 (1974).
- ⁹ Galante, L., Colston, K., MacAuley, S., and MacIntyre, I., *Lancet*, **i**, 985-988 (1972).
- ¹⁰ Larkins, R. G., *et al.*, *Clin. Sci. molec. Med.*, **46**, 569-582 (1974).
- ¹¹ Wasserman, R. H., Bar, A., Corradino, R. A., Taylor, A. N., and Peterlik, M., in *Proc. Vth Parathyroid Conference* (edit. by Talmage, R. V.) (Excerpta Medica, Amsterdam, 1975).
- ¹² Lawson, D. E. M., Smith, M. W., and Wilson, P. W., *Febs Lett.*, **45**, 122-125 (1974).
- ¹³ Corradino, R. A., and Wasserman, R. H., *Nature*, **252**, 716 (1974).

dence), has meant that these drugs can be used as pharmacological tools to study the function of endogenous prostaglandin release in the body (see Ferreira and Vane, *The Prostaglandins*, volume 2, edit. by Ramwell; Plenum Press, New York, 1974 for review).

An alternative way of studying physiological events in the absence of prostaglandins is to actively immunise animals against them; prostaglandins can easily be made immunogenic by coupling them to a carrier protein such as bovine serum albumin. Active immunisation against prostaglandins has recently been shown to afford a certain protection against inflammatory stimuli (Ferreira *et al.*, *Prostaglandins*, in the press). Using similar techniques Horton and Poyser have obtained further evidence that prostaglandin F $_2$ $_a$ is involved in luteolysis in the guinea pig (*Prostaglandins*, **5**, 349; 1974). This experiment adds further support to the idea (now widely accepted) that prostaglandins are involved in luteal regression in several animal species (Pharris, *Perspec. Biol. Med.*, **13**, 434; 1970) though apparently not in man. Other aspects of the reproductive process are also susceptible to modification by prostaglandins (see Persaud, *The Prostaglandins*, volume 2, edit. by Ramwell; Plenum Press, New York, 1974). Prostaglandins are in clinical use for the termination of pregnancy and induction of labour and an interesting corollary to their stimulating action on the myometrium is the manner in which aspirin-like drugs delay parturition in both man and animals (see Persaud, cited above). These effects reinforce the view that endogenous PG production in the uterus is the final pathway for expulsion of the contents.

When Vane made his observation that aspirin inhibited prostaglandin biosynthesis in a cell-free system (*Nature*, **231**, 232; 1971) there was already some evidence indicating that prostaglandins were involved in the pathogenesis of inflammation and fever. Thus, as Vane pointed out, inhibition of prostaglandin biosynthesis could explain the well known anti-inflammatory and antipyretic effects of aspirin. Since then a link has been established between inhibition of prostaglandin biosynthesis and the analgesic actions of aspirin-like drugs (Ferreira, *Nature new Biol.*, **240**, 200; 1972; Ferreira, Moncada and Vane, *Br. J. Pharmacol.*, **49**, 86; 1973). Important features of the aspirin-like drugs are that the analgesia they produce is mild and that they act peripherally (Lim, *Arch. int. Pharmacodyn.*, **152**, 25; 1964). By contrast the narcotic analgesics are extremely potent drugs which act within the central nervous system and do not have any direct effect on prostaglandin biosynthesis. Recently,

Aspirin, prostaglandins, endoperoxides and platelets

from R. J. Flower

DURING the first six months of 1974 almost 500 publications on prostaglandins were cited by *Index Medicus*; this compares with some 400 publications *per annum* two years ago, approximately 170 in 1970, fewer than 100 in 1968, and only 25 in 1966. It is not difficult to understand this tremendous interest in prostaglandins: almost all mammalian cells possess the enzymatic machinery (generally known as 'prostaglandin synthetase') for the synthesis of prostaglandins from precursor fatty acids; furthermore, biosynthesis and release of prostaglandins from cells can be evoked by a wide range of physiological, pharmacological or pathological stimuli, and the different prostaglandins which are produced have a wide spectrum of pharmaco-

logical activity in concentrations often as low as 10⁻⁸M. These properties make prostaglandins obvious candidates for mediators of a wide range of physiological events.

Physiological functions

Enormous impetus has been given to research on their physiological function by the discovery that the 'aspirin-like' drugs inhibit prostaglandin biosynthesis (Vane; Ferreira, Moncada and Vane; Smith and Willis, *Nature*, **231**; 1971). These observations have since received widespread confirmation. The blockade of prostaglandin synthesis by drugs of the aspirin type, being unique, specific, and common to almost all tissues and all species so far examined (see Flower, *Pharmac. Rev.*, **26**, 33; 1974 for evi-

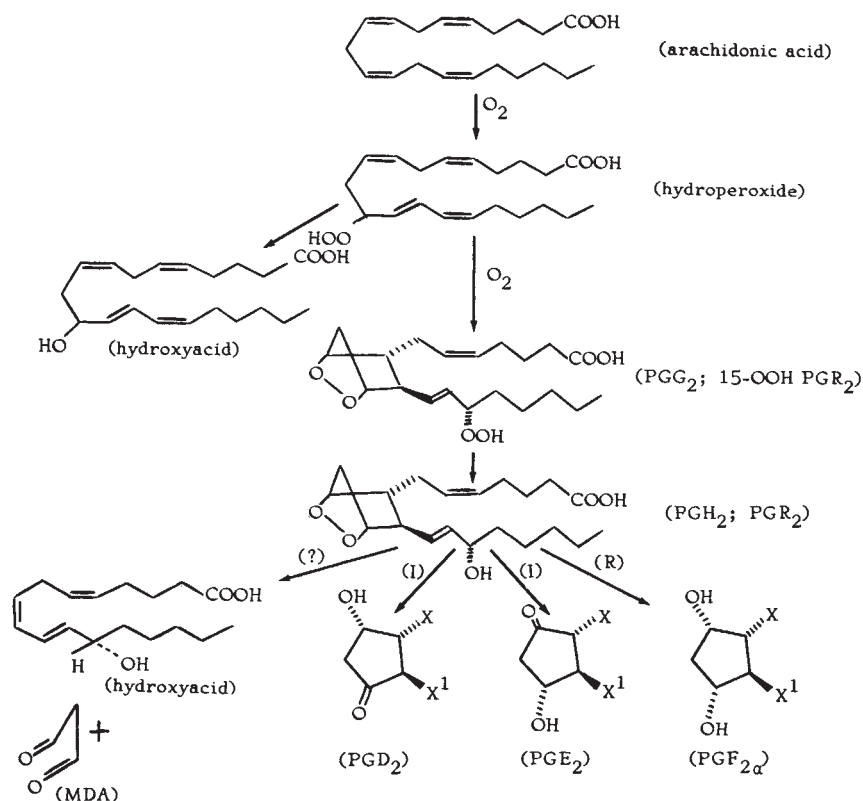


Fig. 1 Proposed reactions for generation of cyclic endoperoxides and prostaglandins from arachidonic acid. This scheme is based largely on the work of Nugteren *et al.*, *Rec. Trav. Chim. Pays-Bas.*, **85**, 405; 1966; Nugteren and Hazelhof, *Biochim. Biophys. Acta.*, **326**, 448; 1973; Hamberg and Samuelsson, *J. biol. Chem.*, **242**, 5336; 1972; *Proc. natn. Acad. Sci. U.S.A.*, **70**, 899; 1973 and Hamberg *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **71**, 345; 1974. Only the ring structures of the prostaglandins have been drawn; X and X' refer to the appropriate hydrocarbon chains. I=isomerisation; R=reduction; ?=enzymic or non-enzymic? MDA=malondialdehyde. Although this scheme shows the conversion of arachidonic acid into G₂, H₂, E₂, and so on, the conversion of di-homo- γ -linolenic acid probably proceeds by an analogous pathway.

however, an interesting and provocative hypothesis by Collier's group (see *Nature*, **248**, 241; 1974; and **252**, 56; 1974) suggests that these narcotic drugs may exert their effects by preventing the stimulation of brain adenylyl cyclase by E-type prostaglandins.

Endoperoxides—the 'intermediates'

One of the most significant advances of the last two years has been the isolation and characterisation of two intermediates in the conversion of precursor fatty acids to prostaglandins. These intermediates are of interest to the biochemist because prostaglandin synthesis is an unusual type of oxygenation reaction. They also interest the pharmacologist, for Gryglewski and Vane (*Br. J. Pharmacol.*, **45**, 37; 1972) have suggested that 'rabbit aorta contracting substance' (RCS—a potent smooth muscle contracting factor first reported by Piper and Vane in 1969 whose release from perfused guinea-pig lungs during anaphylaxis is blocked by aspirin) was an intermediate in prostaglandin biosynthesis. Hence many workers had been anxious to test the biological activity of the intermediates.

The precursors of prostaglandins are the 20-C essential fatty acids. There are several groups of prostaglandins distinguished by the nature and geometry of substituent groups in the ring (for example E, F, D) and the number of double bonds in the side chains (for example E₁, E₂, E₃; F₁, F₂, F₃) all of these can be produced by the synthetase complex from the appropriate precursor. In 1965 Samuelsson proposed the existence of a common intermediate—a "cyclic endoperoxide"—which once formed could break down to either E, F, or D type prostaglandins. Two such endoperoxides have now been isolated by Samuelsson's group (*Proc. natn. Acad. Sci. U.S.A.*, **70**, 899; 1973; *ibid.*, **71**, 345; 1974) and by Nugteren and Hazelhof (*Biochem. Biophys. Acta*, **326**, 448; 1973). The first 'stable' product to be formed by the oxygenation reaction is called by Samuelsson's group 'prostaglandin (PG) G' and by Nugteren and Hazelhof '15-hydroperoxy PGR'; the second intermediate has been named 'PGH' by Samuelsson and 'PGR' by Nugteren and Hazelhof (see Fig. 1). Both the endoperoxide intermediates are very unstable in aqueous solution,

spontaneously decomposing to prostaglandins (half-life approximately 5 min). They can, however, be isolated in some organic solvents and are stable in dry acetone at -20°C for some months.

Their instability in aqueous media has led to difficulties in the testing of the biological activity of these 'pro-prostaglandins'. Generally the endoperoxides have greater smooth muscle contracting activity than the prostaglandins. On the isolated rabbit aortic strip the endoperoxide had a biological activity of 1–2 orders of magnitude greater than those of prostaglandins themselves and initially it seemed as though the activity of the endoperoxides could account for the RCS of Piper and Vane. But a careful comparison of the properties, especially of the breakdown rates of the endoperoxides and of RCS, has made this identity less likely. Thus in addition to the endoperoxides there may be at least one more biologically active intermediate in prostaglandin biosynthesis.

Endoperoxides also seem to have a potent biological action as platelet aggregating agents. In 1973 Willis and Kuhn (*Prostaglandins*, **4**, 127; 1973) reported that preparations of prostaglandin synthetase generated a "labile aggregation-stimulating substance" (LASS). This substance was rapidly generated enzymatically from arachidonic acid (but not from other related fatty acids) and its formation was blocked by aspirin. Early in 1974, a further paper by Willis (*Prostaglandins*, **5**, 1; 1974) described the properties of LASS, observing that it "cannot be distinguished" from the endoperoxides. Almost simultaneously a report by the Swedish group appeared (Hamberg *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **71**, 345; 1974) in which they reported that purified preparations of PGG₂ or PGH₂ were extremely potent platelet aggregating agents in concentrations of 10–300 ng ml⁻¹; furthermore during aggregation of platelets with thrombin, endoperoxide was released in similar concentrations into the medium. Later in 1974 a similar report appeared (Smith *et al.*, *J. clin. Invest.*, **53**, 1468; 1974) confirming and extending these findings. Interestingly, only the endoperoxides from arachidonic acid (that is, the precursors of G₂, E₂, F₂, D₂ and so on) aggregate platelets; the endoperoxides formed from di-homo- γ -linolenic acid (the precursor of G₁, H₁, E₁, F₁, D₁ and so on) do not.

These data help to explain why inhibitors of prostaglandin biosynthesis such as aspirin block platelet aggregation, whereas PGE₂ has only a very weak aggregating action and PGE₁ actually inhibits aggregation. It seems that in platelets the final products of prostaglandin synthetase are relatively inactive end products of the synthetic

reaction and one wonders whether a similar situation obtains in some other systems. Since prostaglandin synthesis (hence endoperoxide generation) can be initiated by damage to cells and tissues, workers from each group have pointed out that endoperoxides are most likely the trigger for platelet thrombus formation at wound sites.

Doses of aspirin which block PG synthetase are (generally speaking) well tolerated, implying that prostaglandin synthetase is not an enzyme vital to the existence of the organism. This accords with the view that prostaglandins are modulators of physiological processes, perhaps mainly involved in local communication between cells, especially in defensive reactions induced by damage or stress. But, because of the exponential increase in prostaglandin literature, a few more years will have to elapse before prostaglandins are assigned a definite role among the body's homeostatic mechanisms.

Ecologists classifying plants

from Peter D. Moore

It is said that when a soldier is confronted by a static object he will be inclined to paint it; a biologist placed in the same situation will attempt to classify it. By the beginning of this century, when the vast bulk of larger animals and plants had been placed into seemingly appropriate taxonomic pigeonholes, a rising breed of biologists, now classified as ecologists, began the formidable task of sorting entire communities of species into meaningful groups. Starting with the belts of climatically determined vegetation on a global scale, they worked their way down to the level of habitats. In Britain this descriptive age culminated in the production of Tansley's *The British Islands and their Vegetation* (Cambridge, 1939), in which vegetation types were defined on simple criteria, mainly the dominant species. On the Continent a more elaborate system was developed by Braun Blanquet in which vegetation types were characterised by plant species which were confined to any given association (so-called 'faithfuls'); these were considered to be of narrower ecological amplitude and therefore more specific indicators of the environment than were the dominants used in the British system. In a modified form, this system remains the standard approach to vegetation description and classification throughout the European mainland.

In Britain the Braun Blanquet system has never really established itself, partly because of the general poverty

Another rock term bites the dust

from L. F. Penny

At last the enigma of the Hesse Till is solved. This, the topmost member of the Devensian (last glaciation) boulder clays on the east coast of England, which most geologists have hitherto regarded as a till sheet in its own right, is now shown by Dr Madgett to be nothing more than the soil profile at the summit of the succession (this issue of *Nature*, page 105).

To be sure, this solution has occasionally been suggested before. Eighty years ago Carvill Lewis suspected it (*Glacial Geology of Great Britain and Ireland*, 209; 1894); Bisat was getting warm in 1940 when he found that the Hesse Till inland (Madgett's Zone C) was different from that on the coast (Zone A), but he was not thinking of a pedological explanation (*Proc. Yorks. geol. Soc.*, 24, 148; 1940); and more recently the same doubt has been expressed by Fenton (*E. Yorks. Field Studies*, 2, 3; 1969). But suspicion is one thing, and proof is another; it is this that is now presented, as a result of the application of modern pedological methods to what was previously regarded as a purely geological problem. Briefly, the so-called Hesse Till in Yorkshire lies sometimes on the Drab Till and sometimes on the Purple Till; and it is now identified, where it overlies the Drab, as weathered Drab; and where it overlies the Purple Till, as weathered Purple. And this is why Bisat's "inland Hesse" "which lies on Drab or directly on Chalk" was different from the "coastal Hesse" (which lies on Purple).

If we ask why such a neat and satisfactory solution has never been proved before, the answer must be that the wrong people have been looking at it. Madgett is a pedologist, who has brought a pedological

eye to the examination of that part of the succession which geologists usually dismiss in their field note-books as "Soil . . . 2 m". The story of the Hesse Till shows how dangerous it is for geologists to draw conclusions about superficial deposits without taking pedological advice (as indeed it would be for a pedologist to interpret a soil profile without an understanding of the geological history of the area). In several countries it is now normal practice for Pleistocene sections to be examined simultaneously by geologist and pedologist, who write it up together; in Britain we are moving toward this more integrated approach, largely due to the activities of interdisciplinary groups such as the Quaternary Research Association, but it is still true to say that much more could be done (and mistakes avoided) by a greater awareness in each group of what the other can contribute.

Clearly, as Madgett suggests, the term Hesse Till should now be dropped. Hesse has never been a good type locality, and if the till there is really weathered Drab, there is no point in perpetuating the name. Perhaps these discoveries will stimulate a more comprehensive revision of the nomenclature of the east coast tills. Are "Drab" and "Purple" really satisfactory as stratigraphical names? Is "Hunstanton" any better than "Hesse", if there too the till is weathered Drab? Probably the time has come to redefine them according to modern usage, in which each lithostratigraphical division is "designated by a distinctive proper name and defined in a specified place or region" (*Int. geol. Correlation Programme*, UK Contribution; Royal Society, 1968).

of our flora and partly as a consequence of the penetrating critique directed at it by Poore (*J. Ecol.*, 43, 226; 1955). Unconvinced about the real existence of plant communities as definable entities, researchers in Britain and in the United States and Australia have preferred to regard vegetation as a continuum of variation in its specific composition. This being so, the most convenient approach to classification has been the erection of reference points within the continuum at appropriate locations (Poore, *J. Ecol.*, 44, 28; 1956). As an aid in this task, the tech-

nique of ordination has been used extensively; in essence this involves the comparison of stands of vegetation on the basis of their floristics and the construction of geometrical, spatial models which can best account for the various similarities and differences observed (for example, Bray and Curtis, *Ecol. Monogr.*, 27, 325; 1957).

Hierarchical classification of stands, reminiscent of the Braun Blanquet type, has found a place in British plant ecology mainly as a result of the work of Williams and Lambert (*J. Ecol.*, 47, 83; 1959), who initially used a chi-

square statistic for stand comparison and later an information statistic either for splitting or for agglomerating samples into the most homogeneous groupings possible (Williams, Lambert and Lance, *J. Ecol.*, **54**, 427; 1966, and Lance and Williams, *Computer J.*, **11**, 195; 1968). These methods have provided a degree of objectivity which is not usually a feature of Braun Blanquet classifications, but whether they provide more information to those concerned with community descriptions has often been questioned (see Frenkel and Harrison, *J. Biogeogr.*, **1**, 27; 1974).

In the United States, Whittaker (*Ecol. Monogr.*, **23**, 41; 1953) provided a new approach to the classification of vegetation. He plotted stands along axes representing environmental factors, such as moisture availability and altitude. By substituting at each point the abundance of a given species in that stand he was able to ordinate species with respect to their ecological requirements and in this way learn something of the species and consequently of the nature of the community. It is something akin to this approach which Grime has recently used (*Nature*, **250**, 26; 1974) in his analysis of grassland communities, but instead of employing environmental factors as axes he has taken physiological and morphological characteristics of the plants themselves. He uses log maximum relative growth rate on one axis and competitive index (defined in terms of morphology and litter production) on a second axis and is thus able to locate any species in relation to these two parameters. He can substitute vegetation stands for species on such axes by using the mean maximum relative growth rate and mean competitive index of the species found within the vegetation sample. Vegetation from different habitats produces clusters of points in various parts of the graph and Grime accounts for these positionings in terms of stress and disturbance factors. In habitats where stress and disturbance are low, species of high competitive index predominate (see Grime's earlier model of species density variation with environmental stress, *Nature*, **242**, 344; 1973). Species of low competitive index are found mainly in stressed and disturbed habitats, those in the former having generally low growth rates and those in the latter having higher ones. Grime's concept of three major determinants in vegetation composition (that is, stress, disturbance and competition) has led him to use a triangular form in his ordinations.

The usefulness of this new approach to the classification of vegetation will depend upon the validity of this tripartite factor model of plant communities in habitats other than grassland. Is it always possible to

discern between these ill-defined categories of stress and disturbance? Is maximum potential growth rate of a species either an adequate measure of normal growth potential or an adequate criterion for the differentiation of stress- and disturbance-adapted species? We must await data from other habitats. Meanwhile it is refreshing to find a system which steps beyond classification for its own sake and seeks to provide a predictive model upon which management and conservation programmes can be based.

Oceanic lithosphere thickens with age

from Peter J. Smith

At active oceanic ridges there is a regional elevation resulting largely from thermal expansion and perhaps in part from phase changes related to thermal state. Some of the uplift here may also be due to an anomalously thick asthenosphere with an abnormally low density near the ridge crest. But outside the immediate vicinity of the spreading axis, where such arguments no longer apply, the case for variation in lithospheric thickness is much less certain. Most theoretical models of seafloor spreading have therefore assumed that the lithosphere moving away from a ridge has a constant thickness.

Experimentally, the range of determined lithospheric thickness is wide, although most values lie between 40 km and 110 km. For example, upper mantle sections derived from surface wave dispersion observations suggest that an oceanic plate is 70 km thick on average, but other estimates of the same quantity range from 40 km to 100 km. For continental regions there are recorded thicknesses up to 100 km in tectonic belts and up to 150 km in stable shields. Using estimates of the flexural rigidity of the lithosphere, Walcott (*J. geophys. Res.*, **75**, 3941; 1970) calculated the thickness of the normal continental lithosphere to be 110 km and that of the oceanic lithosphere to be 75 km or more. Magnetotelluric methods, on the other hand, have given 10 km thickness for the zone of spreading on Iceland and 40 km for the subduction zone below Kamchatka, the latter value having been confirmed by studies of the attenuation of shear waves.

In short, although existing experimental data probably give lithospheric thickness to the correct order of magnitude, they are far from sufficient for testing whether or not the lithosphere produced at any given ridge has a constant thickness. Moreover, as Vogt (*Earth planet. Sci. Lett.*, **23**, 337; 1974) now points out, there is an inherent

danger in comparing directly determined lithospheric thicknesses insofar as different methods do not necessarily define lithospheric plate in the same way. Rheologically, plate thickness is the maximum depth at which brittle fracture can occur; magmatically, it is the minimum depth at which partial melts are ubiquitous; thermodynamically, it could be defined as the pressure-temperature level at which partial melting begins in a system with known or assumed physical properties; and so on. Not only are these definitions not necessarily identical, the lithosphere-asthenosphere boundary may not even be sharp.

For the time being, therefore, direct experimental determinations are of limited use and recourse must still be made to theoretical models. But the problem with theoretical models involving constant lithospheric thickness is that the thickness is assumed rather than predicted by the model itself. In the important model proposed by McKenzie (*J. geophys. Res.*, **72**, 6261; 1967), for example, lithospheric thickness is a free parameter not determined by the physics; and it was to overcome this difficulty that Parker and Oldenburg (*Nature phys. Sci.*, **242**, 137; 1973) put forward a modification which enabled them to predict that, beyond 10 km from a ridge axis, the thickness of the lithosphere should increase in proportion to the square root of age.

To test which, if either, view is correct—the traditional assumption or the Parker-Oldenburg prediction—Vogt has adopted and adapted a method first used by Eaton and Murata (*Science*, **132**, 925; 1960) to determine the minimum depth from which magma must rise to reach the summit of Mauna Loa volcano, Hawaii. Eaton and Murata assumed that at the depth of the magma source the magma column exerts the same lithostatic pressure as the surrounding normal crust and upper mantle, including water and sediment (that is, there is isostatic equilibrium). The source depth thus determined was 57 km; and although the calculation was carried out before the days of plate tectonics, this depth agrees with the lithospheric thickness beneath Hawaii obtained by more modern means (for example, from the level of deepest seismicity). Vogt therefore believes that the magma is formed beneath the lithosphere and that what Eaton and Murata were measuring was, in effect, the plate thickness.

It is implicit in this that volcanoes such as Mauna Loa cease activity because of an isostatic limitation and not simply because no further magma is available. If this argument may be applied to most volcanoes, it then follows that there should be an observable relationship between volcano

height and plate thickness; in other words, it should be possible in principle to determine plate thickness indirectly from volcano height. In practice, this will only be possible for active or recently extinct volcanoes because older volcanoes will have been eroded and their heights lost for ever. Moreover, there are almost certainly bound to be some volcanoes which cease activity because of insufficient magma and others that have not yet reached their full height, and in these cases heights will also be too low to conform with any volcano height-plate thickness relationship.

But what do the data show? From the mathematics of the isostatic system, Vogt concludes that, when corrected for the buoyancy of their submerged bases, the height of young oceanic volcanoes should vary as the square root of crustal age. A plot of the relevant observations confirms this relationship with a surprisingly low scatter of points about the straight line. Because the data obey the expected relationship, they implicitly support the assumptions upon which the relationship was derived. In other words, the results suggest that most volcano heights are indeed limited by isostasy rather than magma shortage. An increase in volcano height thus implies an increase in plate thickness, and a linear relationship between volcano height and the square root of age implies a similar relationship between plate thickness and square root of age. Hence there is support here for the Parker-Oldenburg model.

On the other hand, this does not mean that the constant thickness hypothesis may be dismissed without further consideration. For if plate thickness remains constant with age, the average plate density will increase with age, and this will also affect the height to which a volcano may grow according to isostasy. Vogt shows, however, that the height-age relationship expected in this case is quite unlike that actually observed.

Pulsing X-ray stars

from James Pringle

OF the sixty or so X-ray stars known in our Galaxy and in the Magellanic Clouds only a few have so far been found to pulse regularly. One, the Crab pulsar, has a pulse period of 33 milliseconds, as do its radio and optical counterparts, and is thought to be a rapidly spinning neutron star. The star's large energy output, several thousand times that of the Sun, derives from its rotational kinetic energy; the pulse period is observed to increase on a time scale of about a thousand years.

Another two, Centaurus X-3 and Hercules X-1, pulse with periods of 4.84 and 1.24 seconds respectively, but, although their X-ray luminosities are similar to that of the Crab pulsar, their pulse periods vary somewhat irregularly with no great evidence for overall speeding up or slowing down. Both these sources of X rays are identified with optical stars. It is almost certain that the X rays originate from a compact companion star, too faint to be visible optically, and that mass transfer from the 'normal' star on to its companion provides the source of energy. One strong possibility is that the compact star is a neutron star with a strong magnetic field (about 10^{12} Gauss). Material falling on to such an object glides along the field lines on to the magnetic poles where it yields about 10% of its rest mass energy on striking the stellar surface. The necessary quantity of energy released in such a small area must lead to high enough temperatures to provide X-ray emission. Rotation of the star, with its two X-ray hot spots, provides the pulse. Radiation produced in such a strong magnetic field should be highly polarised—an important observational test of this hypothesis.

An alternative possibility is that the compact object is a pulsating white dwarf star. For a neutron star, the gravitational energy released by the infall greatly exceeds the nuclear energy released by subsequent hydrogen burning. For a white dwarf of a solar mass, hydrogen burning releases 20 times as much energy as the infall, and even more for less massive stars. It has been suggested that this hydrogen burning could feed energy into pulsations of the star; these would then dissipate through shock waves in the stellar atmosphere. Preliminary work suggested that it was indeed possible to heat the white dwarf's atmosphere sufficiently to provide X rays of sufficient energy and intensity. But in a recent, and more detailed, calculation, Katz and Salpeter (*Astrophysical Journal*, **193**, 429–436; 1974) conclude that these previous results were too optimistic and that any realistic calculation will give X-ray emission too low by many orders of magnitude.

The authors consider radial pulsations of the star which, at a specified layer below the photosphere, are assumed to be sinusoidal in velocity. They stress that the peak downward acceleration of such a motion cannot be taken to be greater than the effective surface gravity: this condition has been violated in previous work. Also, because in general the scale height in the atmosphere is much less than the radius of the star, there is an 'acoustic mismatch' (and thus a very weak coupling) between the low stellar eigen-

modes and atmospheric sound waves or shock waves. This mismatch can only be avoided if the luminosity of the star is close to the Eddington limit, in which case radiation pressure almost balances gravity and the atmospheric scale height is comparable with the stellar radius. In this event, however, the inverse Compton cooling (the sharing of energy between photons and electrons in the stellar atmosphere) produced by the stellar luminosity becomes most severe, and the shock heating of the atmosphere is suppressed. Compton cooling has been ignored in previous work and the present authors find it to be very important in damping atmospheric shock waves and removing energy from any shock heated material. These effects strongly inhibit the conversion of stellar oscillatory motion into multi-keV X rays.

The relevance of the above constraints to the non-pulsing X-ray sources is as yet unclear, although the authors do indicate some misgivings about the white dwarf hypothesis as a whole. On the other hand, there has been a tendency among theorists to ignore the possibility of X-ray emission from white dwarfs and it is likely that further progress will be made in this field before very long.

Giant multipole resonances

from P. E. Hodgson

MANY years ago it was found that the cross sections for the radiative capture of protons by nuclei showed broad maxima at energies around 10 to 20 MeV. These persist throughout the periodic table, and accurate measurements showed that the energies of these states is given approximately by $63A^{-1/3}$ MeV.

Goldhaber and Teller (*Phys. Rev.*, **74**, 1046; 1948) explained these resonances as due to collective oscillations of the neutrons against the protons. This accounted both for their universal character and qualitatively for the variation of their energies from nucleus to nucleus. Subsequently a more detailed microscopic theory interpreted them as a quantum-mechanical superposition of particle-hole excitations giving a resonance of an E1 dipole character (Brown and Bolsterli, *Phys. Rev. Lett.*, **3**, 472; 1959; Elliott and Flowers, *Proc. R. Soc.*, **242**, 57; 1957).

Subsequently these dipole resonances were found to be excited in many other reactions, particularly by the inelastic scattering of electrons and protons (*Nature*, **246**, 250; 1973), and detailed analyses of these data allowed many of the properties of the resonances to be determined.

The identification of giant E1 dipole resonances naturally raises the question of the existence of other multipole resonances in nuclei, and over recent years the evidence for some of these has become more convincing. A giant quadrupole resonance several MeV below the giant dipole resonance was found by (e,e') experiments at Darmstadt (Pitthan and Walcher, *Phys. Lett.*, **36B**, 563; 1971) and confirmed by (p, p') analyses at Oak Ridge (Lewis, *Phys. Rev. Lett.*, **29**, 1257; 1972). Further work at Sendai University (Fukada and Torizuka, *Phys. Rev. Lett.*, **29**, 1109; 1972; Nagao and Torizuka, *Phys. Rev. Lett.*, **30**, 1068; 1973) resolved the new resonance into three separate states, and also provided evidence for other resonances at 19 MeV (E3) and 22 MeV (E2) in ^{208}Pb .

The widths of all these resonances are subject to sum rules analogous to the Thomas-Reiche-Kuhn sum rule in atomic spectroscopy, and it was found that these resonances exhaust a large fraction of the appropriate sum rule, confirming their giant resonance character.

The analyses of proton inelastic scattering by von Geramb and colleagues (*Nucl. Phys.*, **A199**, 545; 1973) has provided particularly elegant and continuing evidence for some of the higher resonances: Fig. 1 shows some of their results. This work enables the apparently irregular behaviour of the inelastic proton scattering at back angles from ^{12}C and ^{16}O to be accounted for, and also gives the parameters of several higher order resonances.

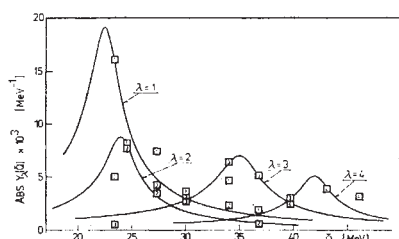


Fig. 1 Multipole resonances in ^{16}O found from analysis of proton inelastic scattering. The numbers in squares and the values of λ are the multiplicities.

All this work has recently been strikingly confirmed by recent measurements of the inelastic scattering of 90 MeV electrons by lead and gold carried out by Pitthan and colleagues at Monterey (*Phys. Rev. Lett.*, **33**, 849; 1974). Some of their results are shown in Fig. 2, and it is clear that the electron energy spectrum has a definite structure that can be

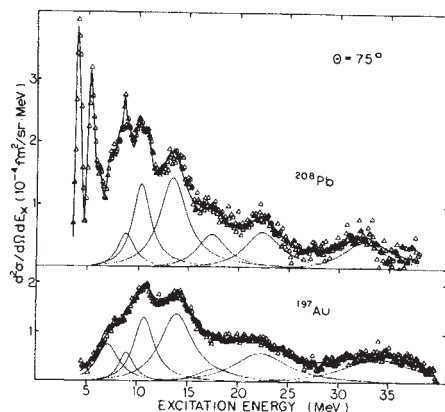


Fig. 2 Spectrum of electrons after inelastic scattering at 75° from ^{208}Pb and ^{197}Au , after subtraction of background. The spectrum is analysed into the components due to the different contributing giant multipole resonances.

analysed into a number of separate components. The energies and widths of these resonances are given in Table 1, together with their multiplicities and whether they are isoscalar ($\Delta T = 0$) or isovector ($\Delta T = 1$), where T is the total isospin. It is notable that the widths of the resonances in ^{197}Au are significantly greater than the widths of the corresponding resonances in ^{208}Pb ; this is to be expected since ^{197}Au becomes dynamically deformed when excited, whereas ^{208}Pb remains spherical.

Of the resonances in the table, the E2 resonance at 10.8 MeV is the familiar dipole resonance. The E3 and E2 resonances at 18 and 23 MeV respectively correspond to those found by the Sendai group.

The lowest E1, E2 and E3 resonances are probably the same as those found by von Geramb and colleagues; the widths are very similar although the energies are shifted somewhat, probably due to a different dependence on A through the periodic table. A distorted wave analysis confirms the E0 character of the resonance observed at 9.2 MeV. This is the first experimental indication of a giant monopole resonance and should make possible a determination of the nuclear compressibility. This identification is supported by evidence from (γ, n) spectra. The resonance at 33 MeV is new and the angular distribution for Au has an E0 (or possibly E2) character. This resonance might be the isovector monopole state proposed by Bohr and Mottelson (*Nuclear Structure*, vol. 2, 1972), but an accurate assignment is difficult.

Table 1 Characteristics of the giant multipole resonances found from analyses of inelastic scattering of 90 MeV electrons by ^{197}Au and ^{208}Pb .

$E_x A^{1/3}$	EL	ΔT	^{197}Au		^{208}Pb	
			E_x	Natural width (MeV)	E_x	Natural width (MeV)
53	E0	0	9.2	2.2 ± 0.5	8.9	1.8 ± 0.5
63	E2	0	10.8	2.9 ± 0.2	10.5	2.8 ± 0.3
81	E1	1	14.0	4.5 ± 0.2	13.6	3.9 ± 0.1
105	E3	0	18.0	5.1 ± 0.7	17.5	4.2 ± 0.7
133	E2	1	23.0	7 ± 1	22.5	5 ± 1
195	E0	1	33.5	10.5 ± 2	33.0	6 ± 1

Persistent superfluid flow

from P. V. E. McClintock

IN a recent communication to *Physical Review Letters* (**33**, 1073; 1974), Galkiewicz and Hallock report the first really satisfying demonstration of a persistent superfluid current in a helium film. In their experiment, which was carried out at the Amherst campus of the University of Massachusetts, they started the ^4He film moving at a velocity of about 5 cm s^{-1} around a closed path some 40 cm in length, and were then able to show that the energy of flow did not measurably diminish over a period of several hours.

Like other liquids, helium tends to form a thin film up the walls of the vessel which contains it, held in place by the Van der Waals attractive force between the helium atoms and the atoms of the wall. In contrast to other liquids, however, whose films usually evaporate a short distance above the free surface and which are constrained by viscous forces to move at very small velocities, the helium film can indulge in superflow, that is, it can flow at several cm s^{-1} without any dissipation of its energy of flow. Thus, any evaporation of the film is quickly replaced by superflow from the bulk liquid. All surfaces in contact with superfluid helium will therefore be completely covered with a relatively thick (typically 200 Å) film of helium. It is superflow in the film which is responsible for the well known and dramatic phenomenon of the beaker of helium which empties itself while remaining upright: the liquid simply syphons itself out through the channel provided by the film.

The non-dissipative nature of film flow has been fairly convincingly established by experiments where a flow from one vessel to another was maintained without any measurable driving force. Yet, apart from some interesting work in compressed powders and other porous media, no previous attempt to set up a persistent flow of the film around a closed path has been successful.

The most impressive of these earlier investigations was probably that of Wang and Rudnick of the University of California at Los Angeles, described in the recently published *Proceedings of the 13th International Conference on Low Temperature Physics* (edit. by K. D. Timmerhaus *et al.*, vol. 1, 239; Plenum Press, 1974). Their substrate consisted of a glass cylinder which could be rotated, and they arranged to be able to measure the times of flight around the cylinder of surface waves on the adsorbed helium films (known as third sound) either with or against

the expected persistent current. The velocities of third sound relative to the substrate in the two directions would have been different in the presence of a persistent flow, but no such phenomenon was observed during a series of very careful experiments. In fact, the authors concluded that any such flow, if it existed, was occurring at velocity of less than 1 cm s^{-1} .

In the Amherst experiment both the geometry and the method of initiating the film superflow were entirely different. Rather than on the outside of a cylinder, the film was made to flow along the inside of a 37 cm length of 1.37 mm diameter stainless steel tube, closed on itself so as to form a continuous loop. Also connected to the loop, by separate short copper tubes, were the annular spaces in two pairs of coaxial cylinder capacitors, c_1 and c_2 . On the 1.2 cm length of the loop between these two connections was wound a heater which, when energised, evaporated the film and prevented it flowing past that point, thus acting as a sort of switch for the superflow. At the beginning of an experiment c_1 and c_2 were partly filled with superfluid helium so that the tubes above them and the stainless steel loop were covered with the film. In coming to equilibrium the liquid levels were, of course, equalised by film superflow. By measuring the capacities of c_1 and c_2 it was possible to determine to a very high precision the amount of dielectric, and thus the level of the liquid helium inside each of them.

To initiate film flow, the heater was energised and a d.c. voltage was applied for a short while across c_2 . The immediate result was a flow of the film out of c_1 , around almost all of the stainless steel loop (except, of course, the short length between c_1 and c_2 which incorporated the heater), and into c_2 . A well known property of film flow between reservoirs is the tendency to overshoot when the levels equalise, resulting in slowly damped oscillations with a period of several seconds. Thus, the disturbance of the levels in c_1 and c_2 was followed by an oscillatory flow between them through the connecting helium film. By de-energising the heater at an appropriate moment the loop circuit was completed for superflow and, though the level oscillations gradually decayed away, it was hoped that a persistent current might be left in the loop.

To test for the presence of a persistent current after a lapse of time, the heater was again energised, thus opening the loop circuit, so that any kinetic energy flow in the film would have to go into depressing the level in the capacitor on the upstream side of the heater and therefore exciting oscillations once more. In practice level

oscillations did indeed occur, even after a lapse of as long as seven hours; and the amplitude of the oscillations did not vary to a measurable extent with the waiting time. The implication must be that Galkiewicz and Hallock had succeeded for the first time in setting up a persistent state of flow around the loop, quite analogous to the persistent electrical current which can be set up in a closed circuit of superconductor: in each case there is a macroscopic flow of particles which apparently persists indefinitely without any dissipation of energy.

Why, on the other hand, was Wang and Rudnick's carefully designed experiment a failure? The reason is not yet known but, on the basis of the present experiment, it is unlikely to be connected with momentum exchange between the flowing film and the helium vapour with which it is in dynamic equilibrium, as had been suggested earlier: this same situation also pertained in the successful Amherst experiment. It is to be hoped that further developments of Galkiewicz and Hallock's new technique will yield the answer to this and other problems connected with film superfluidity.

Changes in enzyme activity during plant photomorphogenesis

from our Plant Cell Physiology Correspondent

PROFOUND changes in the activity of many plant enzymes occur during photomorphogenesis, the response of dark-grown plants to light. Most changes are brought about by red or far-red light acting through the phytochrome system; in some instances a photoreceptor absorbing blue light is involved. Typically the activity of enzymes already present in dark-grown plant tissue is stimulated above the basal level within hours. Although other phytochrome-controlled responses occur more rapidly, this system is very convenient for studying the regulation of enzyme activity during plant development. Using the density-labelling technique several groups have obtained evidence suggesting that at least two mechanisms are involved—*de novo* enzyme synthesis and the activation of pre-existing inactive enzymes.

The essence of the density-labelling technique is simple. In order to test whether an increase in enzyme activity in response to a given stimulus is due to synthesis, a heavy isotope such as ^{18}O , ^3H or ^{15}N is briefly supplied to the

tissue at the same time as the stimulus. A protein extract is later prepared from the tissue and fractionated by buoyant density in CsCl using an ultracentrifuge. If the degree of labelling of the enzyme being studied is significantly greater in the stimulated tissue than in control tissue this indicates an enhanced rate of *de novo* synthesis.

Attridge, Johnson and Smith (*Biochim. Biophys. Acta*, **343**, 440; 1974) have studied the phytochrome-controlled increase in activity of phenylalanine ammonia-lyase (PAL) in mustard cotyledons. They have shown, using $^3\text{H}_2\text{O}$, that PAL and acid phosphatase become density labelled in cotyledons of both irradiated and dark-grown plants, indicating that enzyme synthesis is taking place in all cases. The degree of density labelling of acid phosphatase is unaffected by irradiation, whereas if PAL labelling is measured during the time that the enzyme activity increases in response to light the density labelling of the enzyme is actually lower than in dark grown plants. The authors suggest that the increase in enzyme activity detected in the light is due to the activation of previously synthesised enzyme, although a decrease in the rate of degradation of the enzyme could also be involved. Similar results were obtained for the blue-light controlled increase in PAL activity in gherkin hypocotyls (Attridge and Smith, *Biochim. Biophys. Acta*, **343**, 452; 1974) and the authors cite evidence for the presence of a substance in this tissue which reversibly inactivates PAL.

Another enzyme that increases in activity in irradiated mustard cotyledons is ascorbic acid oxidase. Attridge (*Biochim. Biophys. Acta*, **362**, 258; 1974) has shown that when dark-grown plants are transferred to $^3\text{H}_2\text{O}$ and irradiated with far-red light, the density of the enzyme increases to the maximum observed within six hours (much more rapidly than the dark control). This evidence strongly suggests that ascorbic acid oxidase, unlike PAL, is synthesised more rapidly in response to light. Increased ribonuclease activity in lupin hypocotyls also seems to be due to phytochrome-controlled stimulation of enzyme synthesis according to Acton and Schopfer (*Biochem. J.*, **142**, 449; 1974). These workers were able to demonstrate a greater increase in density of the enzyme in illuminated plants compared to the dark-grown controls. Band-broadening of the enzyme peak in CsCl also provided evidence for the presence of a population of differently deuterated enzyme molecules—to be expected if the density marker takes a significant time to equilibrate with the amino acid pool. The regulatory mechanisms governing the synthesis of these enzymes remain to be determined.

articles

Earliest record of man's presence in Britain

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Middle Pleistocene deposits near Westbury-sub-Mendip have yielded flints which pose problems in the local geological context. Some of the flints show signs of having been worked by man and as such they may be artefacts. The flint-bearing layers contain a rich mammalian fauna of Cromerian age, which suggests that the artefacts may mark the earliest record to date (~400,000–500,000 yr BP) of man's presence in Britain.

WORKED flints have been recovered from cave sediments of late Cromerian age near Westbury-sub-Mendip, Somerset. The deposits, which may provide the earliest record yet of man's presence in the British Isles were first noticed in 1969 (see refs 1 and 2). They are exposed in a working Carboniferous Limestone quarry (ref: ST 506504) between 213 m (700 feet) and 244 m (800 feet) OD, on the southern plateau edge of the Mendip Hills. They infill, to ground level, what is believed to be part of a former cavern system. The present exposure, in the unworked northern face of the quarry, is 90 m wide, and nearly 30 m high.

Stratigraphy

The main stratified sequence¹ (Fig. 1) is no longer visible, and much of it has been blasted out in the interests of safety and has been lost forever. The beds were well stratified and about 10 main layers, grouped into two main divisions, could be distinguished, in which three separate mammal faunas have been recognised (Table 1).

The sands and gravels of the Siliceous Group (Table 1) are waterlaid deposits, comprising material that is largely foreign to the area. Rare, angular fragments of Carboniferous chert occur, but Carboniferous Limestone, if it was ever present, has been removed by solution. The foreign constituents are thought to be derived from former cover sediments, but no flint has been found in these, which emphasises that the latter is foreign to the overlying Calcareous Group. A sparse mammal fauna has been collected from the gravels and although it is apparently derived it is no earlier than late Lower Pleistocene.

The Calcareous Group comprises conglomerates and breccias made up of rounded to angular fragments of Carboniferous Limestone, with variously coloured matrices of silt and clay. Well stratified horizons occur only in the central area of the infill, and these yield both the flint and the richest remains of large fossil mammals. The bulk of the mammal finds have so far come from beds 3, 4 and 5 (Fig. 1), which are regarded as downwash accumulations from a former, upstream 'den'. Unfortunately, it is this valuable stratigraphical sequence which has been destroyed. Laterally to the west of this sequence is the Rodent Earth (Table 1) and on the east side is an extensive sequence of breccia deposits which are

rich in fossil mammal material, but which have not yielded flints so far.

Most of the rock constituents of the Calcareous Group are Carboniferous Limestone, though there are indications that some of the limestone is not local, but has been transported into the infill from the surrounding district. The only other rock types in this group are the flints, and three rounded pebbles of ironstone, all found in the central stratified sequence of the Calcareous Group.

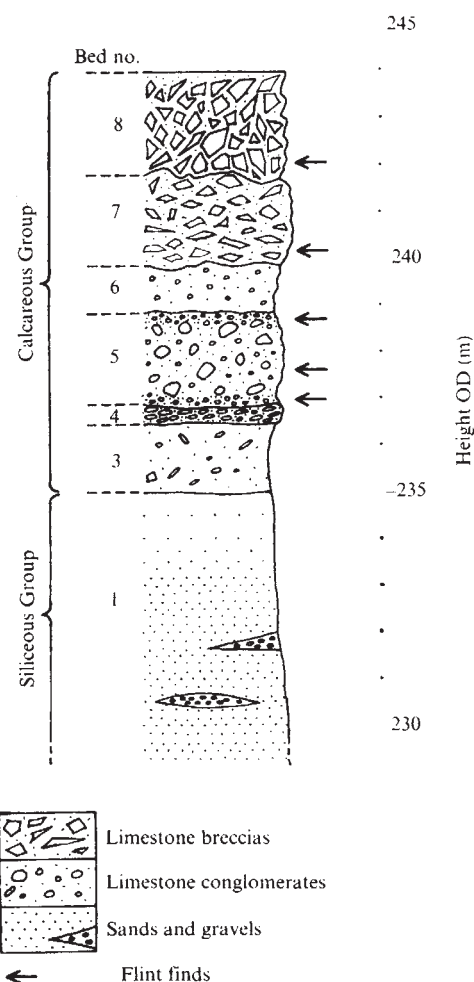


Fig. 1 Stratigraphical section of the upper part of the main layered sequence, visible up to July 1974.

Fauna and age

The mammal faunas have been used to date the deposits and experimental radiometric work on stalagmite samples from Westbury is under way (D. Ford, personal communication).

The fauna of the Calcareous Group comprises predominantly bear remains, probably all *Ursus deningeri* Reichenau, the bones and teeth of which represent individuals of all ages, indicating that the species was a resident of the area. Shelter was probably available in a former upstream, cavern area, which provided a den for bears and occasional living quarters for other hunting animals. A large proportion of other carnivores which are of Cromerian aspect are represented in this fauna, of which the small wolf *Canis lupus mosbachensis* Soergel is very common. Remains of the sabre-toothed cat *Homotherium latidens* Owen, have been found in a number of horizons, and the first British occurrence of the extinct dhole *Xenocyon lycaonoides* Kretzoi, and the 'European jaguar' *Felis gombaszoegensis* Kretzoi, are recorded here. Herbivores form a proportionately small part of this fauna, but they include the rhinoceros *Dicerorhinus etruscus* Falconer which is an important faunal element in indicating a Cromerian age.

Few mammal remains have been collected from bed 8 mainly because it is not easily accessible. The contemporary Rodent Earth is physically separate from the main outcrop of the Calcareous Group, but apparently belongs to it on the basis of its fauna. The fauna of the Rodent Earth comprises predominantly small mammals, most of which are voles, *Pitymys gregaloides* Hinton being the most common. The first British occurrence of the vole *Pliomys episcopalensis* Méhelyi is recorded here. Both of these species indicate that this fauna is pre-Holsteinian (pre-Hoxnian). The abundance of *Arvicola cantiana* Hinton and absence of *Mimomys*, suggests strongly, however, that this fauna is later than the Upper Freshwater Bed of West Runton³ (Cromerian Zone II⁴), as *Mimomys* is believed to be the direct ancestor of *Arvicola*^{5,6}.

Table 1 Main divisions of the early Middle Pleistocene sequence

Calcareous Group (cave conglomerates and breccias)	Rodent Earth Fauna
Siliceous Group (waterlaid sands, silts and gravels)	Calcareous Group Fauna
	Siliceous Group Fauna

The faunas of the Calcareous Group and the Rodent Earth correlate well with the European sites at Mosbach, Mauer, Hundsheim and Tarkö, which are usually considered to be between Cromerian (*sensu stricto*) and Elsterian in age. Correlation with the type Cromerian of the Cromer Forest Bed Series³ is difficult because most of the mammalian material of the type area was collected in the nineteenth century, and records of localities and stratigraphical position are often lacking. There are, however, good records from the West Runton Upper Freshwater Bed⁴. By comparison, the Westbury faunas seem to be younger, and for this reason are here termed 'late Cromerian'. It is possible that they are of about the same age as the younger fauna from Ostend, near Bacton⁴, as *Arvicola cantiana* is common to both sites. Koenigswald⁶ regards the faunas from Mosbach, Mauer, Hundsheim and Tarkö as later than the Upper Freshwater Bed but earlier than Holsteinian (Hoxnian). It would seem that these sites, and the Westbury site as well, show clear faunal distinctions from the Holsteinian, and to some extent from the Cromerian (*sensu stricto*), yet these faunas are themselves temperate. It may be that they are separated from the Cromerian (*sensu stricto*) by a climatic barrier such as a cold phase, or a fully glacial phase (see ref. 7). Preliminary palynological data (R. N. L. B. Hubbard, personal communication) on a Westbury sample from the level of bed 4, indicates that this is zone II of an interglacial. It is hoped that future work on the palynology, fauna

and sedimentology will elucidate the problem of whether a previously unrecognised temperate stage is represented in Britain between the Cromerian (*sensu stricto*) and the Hoxnian.

Flints

Flints occur at five horizons within the Calcareous Group (Fig. 1). The flint is white and usually rotted, often to the extent that it breaks down easily to a fine powder. It is non-calcareous, shows conchoidally fractured faces, and contains

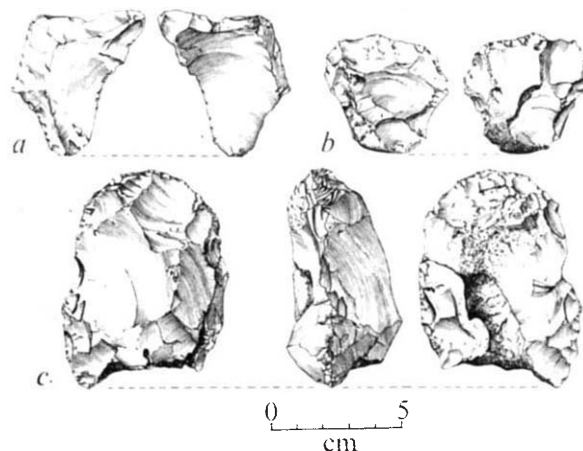


Fig. 2 a, struck flake, from bed 5; b, crude bifacial flint from bed 5, worked from a flattened pebble; c, Ovate, bifacial worked flint from bed 8.

Upper Cretaceous foraminifera. It occurs most commonly as angular or subangular pieces up to 3 cm in size; in bed 5 they occurred as frequently as one in every 8,000 cm³. Sizes between 3 cm and 9 cm are rare (Fig. 2). Despite the chemical breakdown in most of the flints, the larger specimens are well preserved and show few signs of rounding. Even under microscopic examination the ridges between the flake scars are sharp and fresh, indicating that the flints have not travelled far. Only around the leading edges of the large flints do signs of wear and abraded occur.

Five of the flints show apparent signs of human workmanship. The oldest two are from bed 5; one is a flake (Fig. 2a) and the other is a small bifacial implement (Fig. 2b) which retains part of the original surface of the flattish pebble from which it was fashioned. The largest and most diagnostic implement (Fig. 2c) is from bed 8; it is a thick bifacially worked ovate. The two bifacially worked pieces of flint (Figs 2b and c) are probably Acheulian (K. P. Oakley, personal communication). As the associated fauna has been identified as Cromerian, these implements must rank among the earliest Acheulian artefacts known in Britain, presumably comparable with the so-called Abbevillian in the 45 m terrace of the Somme (K. P. Oakley, personal communication). The preservation of the flints, and the fact that sediment samples (from the level of bed 4) broken down for pollen, contained abundant microscopic charcoal fragments (Hubbard, personal communication), indicate that an occupation site may have existed in the former cavern area.

The new finds at Westbury support suggestions⁸ of an early date for the worked flints from the basal breccia at Kent's Cavern, Torquay⁸, because it is now clear that man was present in southern Britain much earlier than had been supposed. Unfortunately, the mammal fauna from these basal breccias is too poorly known at present to allow reliable correlation with the Westbury deposits, though the presence of *Arvicola*

and *Pitymys gregaloides* at both sites is highly suggestive of such a correlation.

The earliest occupation sites in Europe have, from a consideration of their mammal faunas, been described broadly as Cromerian⁹. Those with good artefactual evidence, within a well defined stratigraphy, and with good faunal associations are extremely rare; they include, however, Vértesszöllös in Hungary (considered to be Elsterian in age), and Vallonnet in France⁹. Although Westbury does not seem to represent the immediate area of an occupation, there is evidence that an occupation site may have existed very close to the present location of the artefacts. This, and the fact that Westbury displayed a very well defined stratigraphy with very rich and abundant faunal associations, ranks Westbury among the most important Pleistocene sites in Europe with evidence of man.

Although the valuable flint bearing strata have been blasted

away, it is hoped that more finds will be made by examining all of the fallen material; new flint bearing layers may become exposed as a result of future excavations.

I thank Westbury Quarry (Mixconcrete Aggregates Ltd) for permission to study deposits in the quarry, and Professor D. T. Donovan, Dr J. E. Robinson and Dr A. J. Sutcliffe for criticism. I thank Miss M. O. Miller (British Museum) for drawing the flints.

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Birefringence experiments on isolated skeletal muscle fibres suggest a possible signal from the sarcoplasmic reticulum

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When stimulated by external electrodes, a frog muscle fibre bathed in hypertonic Ringer gives a birefringence signal consisting of three components. The small first component is due to the action potential of the surface membrane, and the much larger second component may be related to voltage change across the sarcoplasmic reticulum associated with the release of Ca²⁺. The third component is of uncertain origin.

SEVERAL investigators^{1–8} have described light signals which can be detected during the twitch of vertebrate skeletal muscle. These signals are usually measured as changes in light intensity, but depend on various optical mechanisms, including absorption and/or scattering^{1–5}, fluorescence^{4,6} and birefringence^{4,7,8}. In the hope of learning more about the very early events underlying the activation of contraction, we have carried out birefringence experiments on single muscle cells. The major problem in optical experiments on excitable tissue is often not so much to find light signals but to identify as precisely as possible the subcellular events which cause them. So far this effort has been less successful in muscle than in nerve.

Cohen *et al.*⁹, in birefringence experiments on the squid giant axon, showed that signal averaging techniques could detect a small decrease in light intensity coincident with the action potential. This signal had a peak size of about 1×10^{-5} (maximum change in light intensity during the spike divided by resting light intensity). Voltage clamp experiments¹⁰ provided good evidence that the birefringence signal was principally related to potential change across the surface membrane, and showed that the light signal followed the voltage change with a time constant of tens of microseconds.

Unlike nerve, a skeletal muscle fibre has in addition to the surface membrane two well developed membrane systems

thought to be important in the activation of contraction. These are (1) the transverse tubular membranes (T-system), which has in frog sartorius muscle 5–10 times as much membrane area as the surface^{11,12}, and (2) the sarcoplasmic reticulum (SR), which has in frog 75–150 times as much membrane area as the surface¹¹. The physiological roles of these membrane systems are, in outline, fairly well understood. The action potential on the surface membrane depolarises the T-system within the fibre^{13,14}, which in turn triggers the release of Ca²⁺ into the myoplasm from its internal storage site, the sarcoplasmic reticulum¹⁵. The increased myoplasmic Ca²⁺ concentration then activates the contractile proteins¹⁶.

If the membrane systems of muscle, like the squid giant axon, give birefringence signals dependent on membrane potential, these signals might be considerably larger in muscle, because of the greater quantity of membrane involved. Carnay and Barry⁴, in birefringence experiments on muscle bundles of about 50 fibres, found a relatively large decrease in light intensity (peak size about 2×10^{-4}) beginning coincident with the extracellularly recorded action potential. As this signal was about 20 times larger than that for the action potential in squid, it seems unlikely that all of it can be attributed directly to depolarisation of the surface membrane. Because all fibres in a bundle or whole muscle will not give the same signal at the same time, we decided to study muscle birefringence signals using the isolated single fibre preparation.

Single fibres from the semi-tendinosus or ilio-fibularis muscles of frogs (*Rana temporaria*) were isolated and stretched around a small vertical glass post, to minimise vibrational motion and hence optical noise, in a glass-bottomed Perspex chamber. One tendon end was attached to the arm of an RCA 5734 mechano-electric transducer for monitoring isometric tension. The chamber was then mounted on the stage of a Reichert Zetopan polarising microscope with a $\times 40$ water-immersion lens (Zeiss, N.A. 0.75) for optically recording from a 500 μm length of the fibre. Two platinum wire electrodes brought close to the fibre delivered the stimulus pulse (duration 0.2–0.5 ms).

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Only those fibres giving an all-or-nothing response to external stimulation were used for experimentation. The illuminating light, from a 100 W FCR tungsten-halogen bulb, traversed a heat filter and two plane polarisers, one preceding and one following the fibre in the light path. To maximise the size of birefringence signals from subcellular structures with optical axes either parallel or perpendicular to the fibre axis, one polariser was oriented at -45° and the other at $+45^\circ$ with respect to the fibre axis. The light detector, a PV-100 photodiode (EG & G Inc.), produced an output current that was a linear function of light intensity transmitted by the second polariser. This output was converted to a voltage signal by means of a conventional operational amplifier circuit, amplified by a 3A9 amplifier in a Tektronix 565 oscilloscope and fed to one of two signal averaging computers, CAT model 1000 (Technical Measurement Corp.) or Nicolet 1072 (Nicolet Instrument Corp.). The response of the light-recording system to a step of light was 70% complete within 80 μ s. A standard Ringer solution was used: 120 mM NaCl, 2.5 mM KCl, 1.8 mM CaCl_2 , buffered with 2.15 mM Na_2HPO_4 and 0.85 mM NaH_2PO_4 to a pH of 7.1 (ref. 17). For many experiments the Ringer was made hypertonic (see below) by increasing the concentration of NaCl. The temperature for most experiments was between 19° and 24° C. In voltage-recording and current-passing experiments, glass microelectrodes with a right-angle bend to fit under the microscope objective were used, in order that the optical signal could be recorded simultaneously with intracellular electrical measurements.

Birefringence signal without fibre movement

During a twitch in normal Ringer, gross movement of the fibre gives rise to very large optical signals, of the order of 10^{-2} even in highly stretched fibres. Bathing a fibre in 2.2T hypertonic Ringer (2.2 times the standard tonicity), however, greatly reduces or eliminates fibre movement while the surface membrane still gives normal or nearly normal action potentials¹⁸. In fibres in which 2.2T hypertonic Ringer reduced the twitch tension response essentially to zero, three components of the optical signal were usually seen, as Figs 1 and

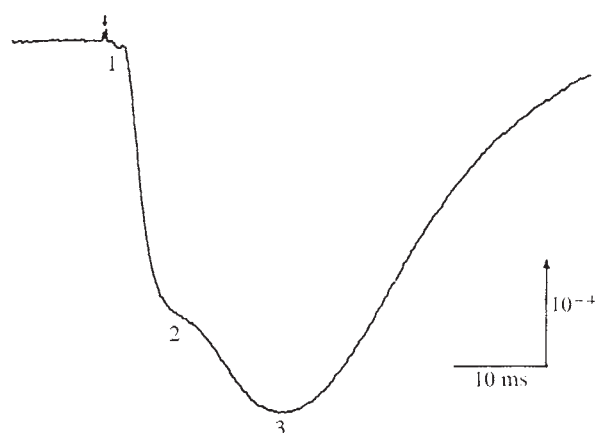


Fig. 1 Three-component birefringence signal in response to external stimulation from a muscle fibre in 2.2T hypertonic Ringer (270 mM NaCl). The trace is an average of 100 all-or-nothing responses. The peaks of the three components are labelled; the first component begins after a small conduction delay following the stimulus artifact (arrow). The vertical calibration gives the ratio of the change in light intensity to the resting light intensity, normalised with respect to a single sweep. A deflection in the downward direction represents a decrease in light intensity. Optical signal d.c. coupled; optical recording 2 mm from the stimulus cathode; fibre diameter 85 μ m.

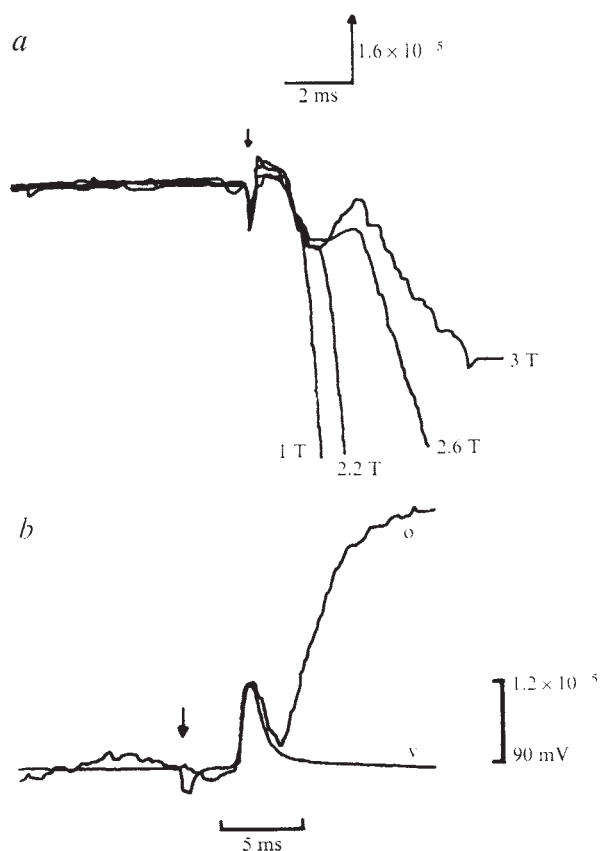


Fig. 2 *a*, Optical recordings from the same fibre in Ringer solutions of various tonicities superimposed by lining up the stimulus artifact (arrow). 1T, 120 mM NaCl; 2.2T, 270 mM NaCl; 2.6T, 320 mM NaCl; 3T, 370 mM NaCl Ringer. Each tracing is the average of 64 sweeps, which were taken in the order of increasing tonicities. Following the record in 3T Ringer, the fibre still gave an all-or-nothing response when returned to normal Ringer, with its average for 64 sweeps falling between the 1T and 2.2T tracings shown. Optical recording 1.5 mm from stimulus cathode; optical signal a.c. coupled; fibre diameter 85 μ m. *b*, Optical (o) and intracellular voltage (v) recordings taken simultaneously in 3T Ringer (370 mM NaCl). The optical tracing has been inverted and scaled to facilitate comparison with the action potential. The upper vertical calibration refers to the optical trace and the lower to the potential trace, each of which is an average of 300 sweeps. Resting potential of the fibre varied between -70 at the beginning of the average to -65 at the end. In agreement with results reported by others, we found that strongly hypertonic solutions tended to depolarise the fibres. Fibre diameter 65 μ m; 7 mm from the stimulus cathode; optical a.c. coupled, voltage d.c. coupled.

3b show: (1) a small, early shoulder which occurred just as the optical trace departed from the baseline and which reached a distinct peak (Fig. 1) or a plateau (Fig. 3b) in 1–2 ms (this component is too small to be distinguished in a single sweep, and in a bundle or whole muscle would probably be difficult to resolve, even with signal averaging, because of differences in conduction velocity among fibres); (2) a relatively large signal, which reached a plateau (Fig. 1) or a distinct peak (Fig. 3b) 5–7 ms after the beginning of the optical signal (large enough to be seen easily in a single sweep); and (3) a more slowly developing signal which reached a peak 15–50 ms after the stimulus. As determined in other experiments in hypertonic Ringer, the first and second components propagate with a velocity appropriate to a muscle action potential at room temperature (about $1.5\text{--}2\text{ mm ms}^{-1}$) and were seen in fibres with a twitch tension response less than 0.3 g cm^{-2} . These signals were primarily due to a birefringence mechanism, since they could be converted from a decrease in light intensity to an

increase in light intensity by adding an appropriate amount of optical retardation in series with the light path using a compensator⁹.

First component

An early shoulder (first component) similar to that of Figs 1 and 3b was usually seen with signal averaging in hypertonic Ringer but only occasionally seen in normal Ringer. An explanation for this is that hypertonicity slows the development of the second component while leaving the first relatively unchanged. Thus the two components become separated in time. This is shown in Fig. 2a, in which optical recordings from the same fibre in Ringer solutions of increasing tonicities have been superimposed by lining up the stimulus artefact. In normal Ringer there is an extremely fast departure of the optical trace from the baseline with no evidence of an early shoulder, whereas in 3T Ringer an early signal about 1.6×10^{-5} (peak size) with a time to peak of 1 ms is revealed. In Ringer solutions of intermediate tonicities, the first component is seen at intermediate states of development, before being obscured by the rising phase of the second component.

It is interesting to know the timing of the first component in relation to the surface action potential. This was determined for the fibre of Fig. 2b in 3T Ringer by simultaneously recording the optical and intracellular voltage signals for an average of 300 sweeps. The vertical gains have been adjusted to match peak size and the optical trace inverted to facilitate comparison. The two traces superimpose very closely until late in the falling phase of the action potential. It seems reasonable therefore to attribute most of this early distinct signal and the shoulder seen in less hypertonic Ringer to the voltage change across the surface membrane.

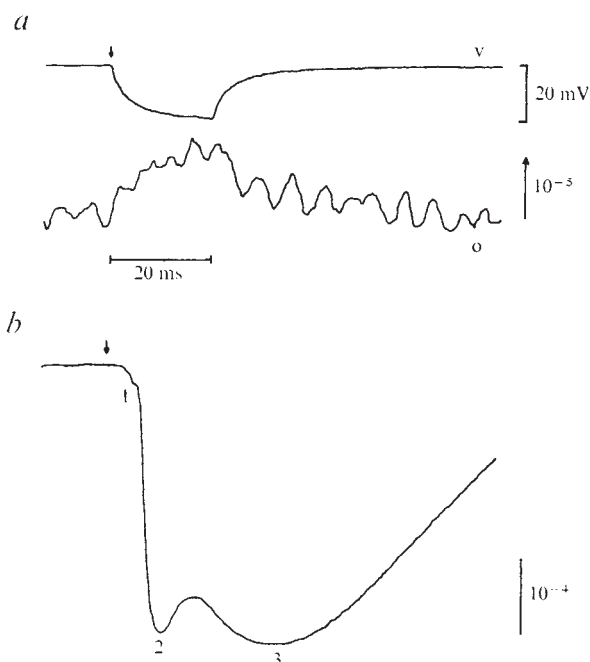


Fig. 3 *a*, Current-passing experiment showing the simultaneously recorded optical (o) and intracellular voltage (v) responses in 2.2T Ringer (270 mM NaCl) for an average of 256 sweeps per trace. At the time indicated by the arrow a 20-ms step of inward current was passed across the cell membrane through a second intracellular electrode (current trace not shown). Resting potential of the cell was -80 mV. Fibre diameter $90 \mu\text{m}$; optical a.c. coupled, voltage d.c. coupled. *b*, Three-component signal in response to external stimulation, taken just before the fibre was impaled for the current-passing experiment shown in (*a*). Optical a.c. coupled; 8.5 mm from cathode; 64 sweeps. The same time calibration applies to both parts of the Figure.

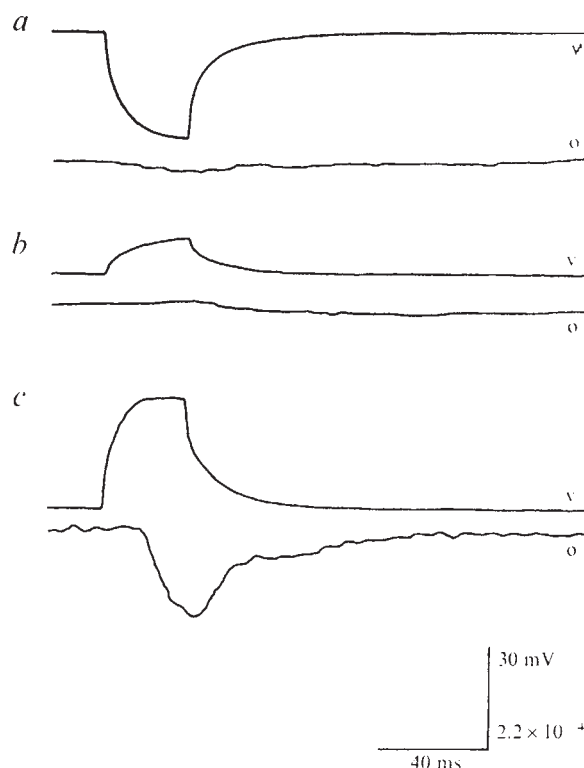


Fig. 4 Current-passing experiment in 2.2T hypertonic Ringer with TTX added (10^{-8} g ml $^{-1}$). Voltage (v) and optical (o) responses were simultaneously recorded during (*a*) a 30 mV hyperpolarising step; (*b*) a 10 mV depolarising step, and (*c*) a 30 mV depolarising step. Resting potential was -68 to -66 mV. Fibre diameter $90 \mu\text{m}$; optical a.c. coupled, voltage d.c. coupled; 32 sweeps per trace.

Second component

In contrast to the first component, the characteristics of which appear to be relatively independent of tonicity, the appearance of the second component is strongly dependent on tonicity. As the tonicity is raised, time to peak is increased and rate of rise (Fig. 2a) and peak size are reduced. For example, the time to peak was often extended to 8 or 10 ms and peak size typically decreased by a factor of 5–10 in going from 2.2T to 3T Ringer. On the other hand, in measurements in normal Ringer directly over the site of action potential initiation, a much larger decrease in light intensity began immediately after the stimulus, occasionally starting with a distinct first component, and reached a plateau or peak 2–5 ms later. In normal Ringer the second component had a peak size of $1\text{--}3 \times 10^{-3}$, about 50 times larger than the second component seen in 3T Ringer.

Since fibre movement itself is a strongly graded function of tonicity, the question of whether the second component is related to fibre movement should be considered carefully. Clearly the second component is not due to simultaneous movement of the whole fibre, because it propagates with the speed of the action potential. When the Ringer was made sufficiently hypertonic so that twitch movement was essentially eliminated, the second component propagated throughout the fibre. In normal Ringer, however, gross fibre movement begins well before the action potential can propagate over all of the fibre. In this situation the propagating second component became obscured by the movement artefact after travelling some distance from the stimulus cathode. With signal averaging in normal Ringer, latency relaxation (the earliest fibre movement detectable by the 5734 transducer) was seen to begin about 2 ms after the stimulus. By this time the second component recorded

adjacent to the stimulus cathode was near or at its peak in many fibres, which also confirms that the second component is not due to gross fibre movement. The second component therefore reflects a propagating change beginning just after the surface action potential, possibly a step linking excitation to contraction.

To determine what fraction of the second component might be attributable to depolarisation of T-system membranes alone, current-passing experiments of the type illustrated in Fig. 3 were done. Figure 3*a* shows the optical signal resulting from a current-passing experiment on a fibre in 2.2T Ringer. The fibre was impaled with two separate electrodes, one for passing current and one for recording potential. At the time indicated, a step of hyperpolarising current was passed across the cell membrane and the voltage and optical responses were recorded and averaged. Although the signal-to-noise ratio was rather poor because of the small signal and mechanical vibrations arising from the two intracellular electrodes, the resulting optical signal for an 18 mV hyperpolarisation had a peak size of about 1×10^{-5} . Although not convincingly resolved in Fig. 3*a*, the time-course of the optical signal recorded in other experiments giving larger voltage and optical responses followed voltage and not current. Since the current step was sufficiently long to ensure that the potential across the T-tubular membranes was within a few per cent of the surface potential, the optical signal in Fig. 3*a* probably reflects voltage changes across both surface and tubular membranes. On this basis, one would not expect an action potential in the T system, causing a tubular membrane potential change of perhaps 100 mV¹⁹, to produce a decrease in light intensity for this cell larger than about 5 to 6×10^{-5} . Therefore, T-tubular depolarisation alone would explain only a small fraction of this fibre's second component, which had an amplitude of 3.5×10^{-4} in 2.2T Ringer (Fig. 3*b*).

Not all current-passing experiments produced a birefringence signal with the polarity indicated in Fig. 3*a*, the polarity to be expected if T-tubular membrane always gives an optical signal with the same sign as that from the surface membrane. Fibres of larger diameter occasionally gave signals of opposite polarity (a decrease in light intensity in response to hyperpolarising steps and an increase in light intensity in response to small depolarising steps), while the first and second components in response to external stimulation showed the usual decrease in light intensity. Although this phenomenon is not completely understood, the results from the current-passing experiments in both kinds of fibres in both 2.2T and normal Ringer support the conclusion that the mechanism underlying the second component is not primarily potential change across the membranes of the T-system.

To investigate further the optical signal resulting from a controlled change of surface and tubular membrane potential, current-passing experiments were carried out using tetrodotoxin⁶ (TTX) (10^{-6} g ml⁻¹), which eliminates regenerative depolarisation of surface and tubular membranes. Figure 4 shows such an experiment for a fibre in 2.2T hypertonic Ringer. At the lower optical gain used, little or no light signal was seen during a current step in the hyperpolarising direction (Fig. 4*a*) or a small current step in the depolarising direction (Fig. 4*b*). A large birefringence signal developed, however, with a moderate-sized depolarisation into the voltage range where contraction is normally activated (Fig. 4*c*). The highly nonlinear dependence of this signal on membrane potential makes it improbable that the signal can be explained simply in terms of voltage change across surface and T-tubular membranes. In other TTX experiments, using larger depolarising steps of short duration, the optical signal was larger and reached an earlier peak, resembling in size and time course the second component seen in 2.2T Ringer following the action potential. This similarity suggests that the second component is caused by the mechanism that gives rise to the optical signal illustrated in Fig. 4*c*, and therefore that the second component is caused by a mechanism which is strongly regulated by surface or T-tubular membrane potentials in the contractile range.

Second component and sarcoplasmic reticulum

The second component is $3\text{--}5 \times 10^{-4}$ in 2.2T Ringer (about 30 times the size of the optical signal attributed to the surface action potential) and in normal Ringer is 1 to 3×10^{-3} (about 150 times that of the surface action potential). If it originates directly from a membrane potential change, only the sarcoplasmic reticulum would seem to have sufficient membrane area to account for its size. Furthermore, it is reasonable to speculate that there is an SR potential change occurring at the time Ca^{2+} is released to trigger the activation of contraction. For example, if Ca^{2+} release were the result of a Ca^{2+} -specific permeability increase in the sarcoplasmic reticulum membrane, the SR might undergo a sizeable potential change within a few ms of the action potential on the surface membrane. During a twitch at room temperature, a muscle fibre typically begins the rapid production of positive tension 3–6 ms after initiation of the action potential. Just before this, a large amount of Ca^{2+} presumably moves across the SR membranes into the myoplasm. Therefore the early timing of the second component, with a time to peak in normal Ringer of 2–5 ms after the surface action potential, is also consistent with the speculation that the second component is signalling an SR potential change associated with the release of Ca^{2+} . (The second component is similar in several ways to a fluorescence signal recently found by Bezanilla and Horowitz⁶ in whole frog muscle and also suggested by them to arise from an SR potential change).

Along these lines, a rough estimate can be made of the magnitude of this possible SR potential change, by using the signal from the surface membrane as a calibration. The calculation assumes that for a given quantity of membrane, either in the SR or on the surface, equal changes in light intensity are produced by equal changes in membrane voltage. (This assumption may be approximately correct, in that both membrane systems, considered as subcellular birefringent structures, involve cylinders of membrane extending the width of the fibre and oriented in the direction of the fibre axis.) Experiment has shown that for a range of fibre sizes the factor relating the peak size of the second component (determined in normal Ringer) to the peak size of the first component (determined in hypertonic Ringer) averages about 1.5 times the factor relating the quantity of SR membrane to the quantity of surface membrane. This latter factor has been determined for frog sartorius muscle to be about $2.7a$, where a is fibre radius measured in microns¹¹. The above assumption then implies that the magnitude of the SR potential change may be about 135 mV (1.5 times the surface action potential in 3T Ringer, which is measured to be about 90 mV).

If the ionic current causing this presumed SR potential change was primarily carried by Ca^{2+} moving from one side of the SR to the other, it is interesting to note how much increase in total myoplasmic Ca^{2+} would correspond to a 135 mV potential change. From the quantity of SR membrane found per litre of muscle¹¹ and the relationship $q = -cV$, the increase is about $35 \mu\text{mol}$ of Ca^{2+} per litre of myoplasm. (For this calculation, a capacitance of $1 \mu\text{F cm}^{-2}$, a value typical of many biological membranes, has been assumed.) Because some Ca^{2+} may also cross the SR in addition to that flowing as net ionic current, $35 \mu\text{mol l}^{-1}$ represents a minimum increase in total myoplasmic Ca^{2+} . This roughly calculated quantity is therefore in reasonable agreement with the total Ca^{2+} thought to be released from the SR during a twitch, $100\text{--}150 \mu\text{mol l}^{-1}$ (M. Endo and L. L. Costantin, personal communication). It is unclear why the peak size of the second component is a graded function of tonicity, falling by a factor of about 50 in going from normal Ringer to 3T Ringer. If the above speculation is correct, the observation is of interest since it would suggest that the amount of Ca^{2+} released during the twitch of a vertebrate fibre is a graded function of tonicity.

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letters to nature

Silicate absorption at 18 μm in two peculiar infrared sources

Two broad emission features often observed in the infrared spectra of cool stars near 10 and 18 μm are commonly attributed to thermal radiation by silicate dust grains¹. This identification is suggested by laboratory analyses of silicate minerals, which indicate strong lattice vibrations resulting from Si–O stretching modes (in the 10- μm region) and Si–O–Si bending modes (near 18 μm). For luminous sources shining through cold, intervening dust clouds which contain silicates, it is expected that both the stretching and bending bands should be observable in absorption (for sufficiently large column densities of dust). The infrared spectrum of the Galactic centre shows a deep absorption feature at 10 μm (N. J. Woolf, unpublished) and evidence for absorption at 18 μm (ref. 2). Strong 10- μm absorption is also observed for the Orion point source 'BN' (ref. 3), IRS5/W3 (ref. 4), and AFCRL809–2992 (ref. 5); no data demonstrating the presence of 18- μm absorption for these sources at longer wavelengths have yet appeared. We report here the detection of 18- μm absorption features in the spectra of AFCRL809–2992 and an infrared object associated with the microwave source OH 26.5 $\pm$ 0.6 (ref. 6). We attribute these features to the Si–O–Si bending mode in silicates.

The object AFCRL809–2992 is a strong infrared source found in the direction of the Cygnus X radio complex. Molecular line emission from HCN and CS has been detected by Morris *et al.*⁷; the weak radio continuum flux of this source (see ref. 5) indicates the absence of a compact H II region and places this object in the Class I category⁷. At 10 μm AFCRL809–2992 is apparently point-like (see ref. 5). OH 26.5 $\pm$ 0.6 is an exceptionally strong OH satellite-line emitter with a radio spectrum which is similar in appearance to that of the Type II OH/IR stars⁶. The near-infrared properties of this source, as well as spectrophotometry of the 10- μm absorption feature, will be reported elsewhere (G. Neugebauer, in preparation). Our raster scans of OH 26.5 $\pm$ 0.6 indicate that the associated infrared source is point-like at a wavelength of 20 μm .

Multifilter infrared photometry of these two objects was obtained with the 2.24-m telescope of the Mauna Kea Observatory between June and September, 1974. Neither source has an optical counterpart, so we located both by scanning the telescope for peak infrared signal. Standard beam-switching techniques were used; the focal-plane aperture was 13'' and

the beam displacement was 19'' in declination. Observation were made with intermediate bandwidth filters over the spectra range 5–33 μm . At wavelengths between 5 and 20 μm , we used stars which have been observed already^{8,9}, to establish an absolute flux calibration. At 25 μm and 33 μm we normalised to α Boo and α Ori, respectively; the calibration at 33 μm has been discussed in greater detail elsewhere¹⁰.

Our observations are summarised in Fig. 1 along with published spectrophotometry of the AFCRL source⁵. The observational uncertainties in the photometry are estimated to be 10% or smaller. Clearly, the spectra of both objects show pronounced absorption features near 10 and 18 μm . Our photometry of the OH source does not delineate sufficiently the 10- μm band to show the deepest (central) part; the observations of the AFCRL source indicate, however, that maximum absorption occurs near 9.8 μm . Similarly, we cannot locate precisely the position of maximum absorption at longer

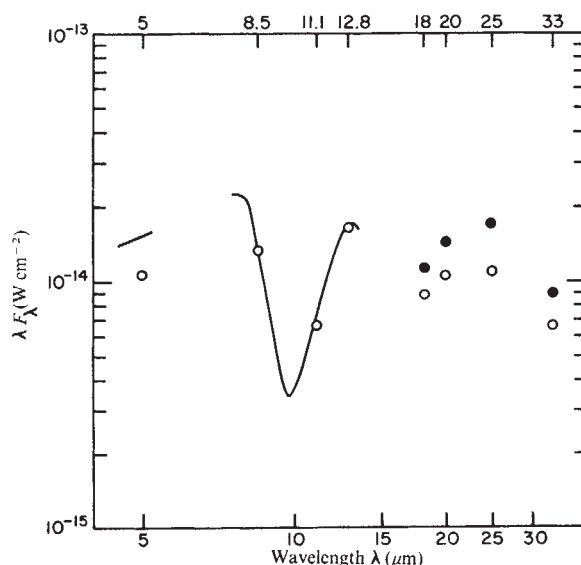


Fig. 1 Observations of two peculiar infrared sources. ○, Photometry of OH 26.5 $\pm$ 0.6; ●, photometry of AFCRL809–2992; —, spectrophotometry of the AFCRL source from ref. 5.

wavelengths, but we infer from the spectrophotometry of the AFCRL source that band centre must lie between 16 and 18 μm . (If the position of the band centre actually falls short of about 16.1 μm , then it will be unobservable from the ground because of absorption by the Earth's atmosphere.) The observed positions of the absorption features are in reasonable agreement with the computations of Knacke and Thomson¹¹, which predict that the Si-O stretching band falls between 9.1 and 9.5 μm and that the Si-O-Si bending band lies between 16.7 and 18.5 μm .

An estimate of the optical depth of the 18- μm absorption band of the AFCRL source can be obtained from a model similar to that discussed by Merrill and Soifer⁵; that is, an emitting source, with a spectrum which follows a Planck curve of $T \sim 350$ K, is assumed to lie behind a cold, absorbing dust cloud. If the Planck curve is normalised to the observed fluxes at 5 and 33 μm , at which wavelengths the opacity of the overlying dust is reduced substantially from the peak near 10 μm , then the optical depth of the cloud at 18 μm is ~ 1.0 . The optical depth at 10 μm for this model is approximately 2.6, giving the ratio $\tau_{18 \mu\text{m}}/\tau_{10 \mu\text{m}} \approx 0.38$. Although the derived optical thickness of the cloud depends on the source spectrum adopted, the opacity ratio is somewhat less sensitive to this assumption. For example, if a smooth curve is drawn through the flux peaks at 5, 8 and 25 μm to represent the intrinsic energy distribution of the emitting source, the ratio $\tau_{18 \mu\text{m}}/\tau_{10 \mu\text{m}} \approx 0.35$ can be derived. The calculations of extinction cross sections for lunar silicates¹¹ yield the range 0.35–0.55 for the extinction ratio, which is in good agreement with the estimates discussed. We conclude, therefore, that the present observations of the position of the 18 μm absorption, and its strength relative to the 10- μm absorption, provide strong evidence to support suggestions that the absorbing agent is silicate dust.

The infrared flux received at the Earth from the AFCRL source at wavelengths between 2 and 33 μm is $3.3 \times 10^{-14} \text{ W cm}^{-2}$; approximately 20% of that energy falls within the 20–33- μm band. If the source is located at a minimum distance of 2 kpc (ref. 5), as suggested by the molecular line emission at radio wavelengths, then the bolometric luminosity of the object exceeds $4.0 \times 10^4 L_{\odot}$. Similarly, the flux measured for OH 26.5+0.6 between 5 and 33 μm is $2.1 \times 10^{-14} \text{ W cm}^{-2}$. The distance to that source is unknown; however, assuming a nominal value of 500 pc (the distance to the Orion Nebula) a luminosity greater than $1.6 \times 10^3 L_{\odot}$ is obtained. For comparison, the total luminosity of the Orion point source is estimated to be $1.5 \times 10^3 L_{\odot}$ (ref. 12).

By considering the observed infrared flux and distance of the Orion source, Becklin *et al.*¹² have concluded that BN cannot be simply a normal, luminous star hidden behind a dense interstellar dust cloud. The strong infrared signals and spectral similarity to BN of the two objects studied here imply a similar conclusion for AFCRL809–2992 and OH 26.5+0.6. Observations at higher resolution from 1 to 33 μm would undoubtedly be of importance in clarifying the relationship of these sources to objects such as BN and IRS5/W3, both of which are likely to be protostars still enshrouded by remnant dust shells.

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Recent increase in Jupiter's decimetric radio emission

DURING an observational programme in support of a study of the linear polarisation properties of Jupiter's decimetric radio emission, it was noted that the planet's total intensity had increased between late summer 1973 and July 1974. The increase is small ($\sim 5\%$) but it seems to be a significant change in the trend of the past decade, during which time Jupiter's decimetric emission has decreased by 20–30% (refs 1–3) with respect to the flux densities observed before 1967.

The measurements were made at Goldstone, California, with the NASA 64-m parabolic antenna operating at 13.1 cm wavelength (2,295 MHz). Jupiter was observed on four nights between July 16 and September 11, 1973, and again on four nights between July 3 and August 17, 1974. The receiving system consisted of a rotatable waveguide polariser, a low-noise travelling-wave maser, and a noise-adding radiometer⁴ that provided stabilisation against short term gain variations. The linear polarisation parameters were determined from consecutive off-on-off measurements of Jupiter made with the polariser set to four different position-angle values at intervals of 45° . Antenna pointing errors were measured and corrected at regular intervals and the system gain was calibrated throughout each night by observing at least three of the radio sources 3C17, 3C48, 3C138, 3C286 and NGC7027. The flux densities of the calibration sources were subsequently determined relative to the radio galaxy Virgo A which has a flux density⁵ of 140.3 Jy at 2,295 MHz.

Jupiter's total intensity was computed for each set of four off-on-off measurements. The data are plotted as a function of the longitude of the central meridian (System III 1957.0) in Fig. 1. Both data sets exhibit the familiar periodic intensity variation with longitude (the beaming curve) but the 1974 data points are consistently above the corresponding 1973 values. The offset is several times greater

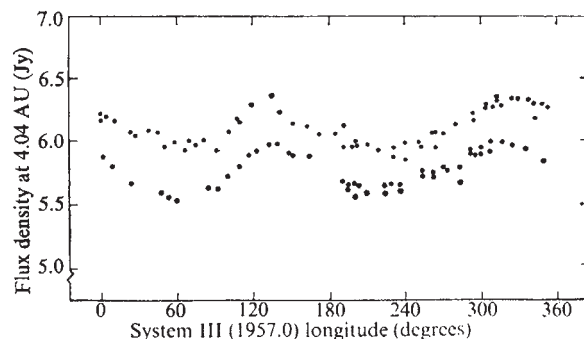


Fig. 1 The flux density of Jupiter at 13.1 cm is plotted as a function of System III (1957.0) longitude. ○, data taken in 1973; ●, data taken in 1974.

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than the uncertainty in the measurement caused by calibration discrepancies or by the effects of background radio sources near Jupiter's position in the sky. So I attribute the difference in flux density to an increase in Jupiter's decimetric radio emission.

It would be incorrect to measure the increase in the decimetric emission simply by determining the offset of one complete beaming curve with respect to the other because the shape of the observed curves varies as a function of D_E , the Jovicentric declination of the Earth⁶. But the strengths of the intensity peaks, which occur twice each rotation when the line of sight from Earth lies in Jupiter's magnetic equatorial plane, are expected to be independent of D_E . The flux densities near these two peaks were determined by fitting second order polynomials to the data with longitudes within $\pm 30^\circ$ of the peaks. The maximum flux densities in 1973 are 5.98 Jy and 5.99 Jy for the first and second peaks shown in Fig. 1. The corresponding values for 1974 are 6.35 Jy and 6.34 Jy. The average increase is 0.36 ± 0.06 Jy; the quoted uncertainty is chiefly the result of uncertainty in the relative calibration of the system from 1973 to 1974.

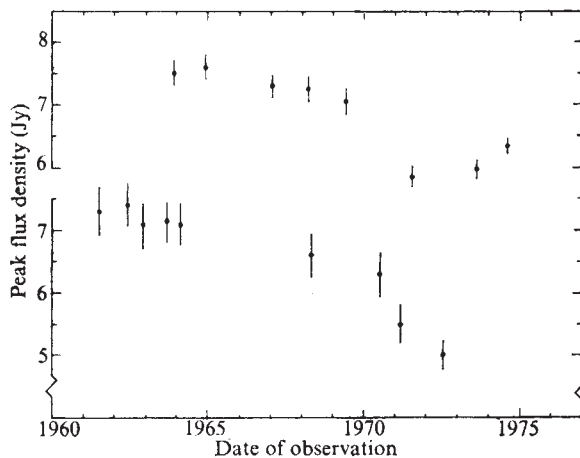


Fig. 2 Jupiter's peak flux density, normalised to a distance of 4.04 AU, is plotted against the date of the measurement. ●, Measurements at 11.1 cm (refs 7–9), 12.6 cm (ref. 1) and 13.1 cm (ref. 6); ○, a collection of measurements at 21 cm as assembled and published by Berge (ref. 3).

In Fig. 2 the average peak flux densities measured at wavelengths of 21 cm and 11–13 cm are plotted as a function of the epoch of each observation. The error bars, which range between 2 and 5%, are estimates of the relative standard errors. The flux density in both wavelength intervals seems to have decreased monotonically until mid-1972. The two 13-cm measurements reported here are the first to show a significant reversal of this downward trend and they demonstrate that Jupiter's decimetric flux density is variable with time scales as short as one or two years.

It is generally believed that the variations are related to changes in the properties of the Jovian Van Allen radiation belts; but specific causes of the variability have not been determined. Attempts to identify periodic variations that might be related to the 11-yr cycle of solar activity or the 12-yr period of the Jovian year have been unsuccessful^{1–3}. Reports of pronounced intensity variations occurring within weeks or months have been published. The most recent of these reports⁷ described variations as great as 30% occurring within a few days in 1968. In contrast, several high precision measurements made with the CSIRO 64-m antenna indicated that the Jovian flux at 11 cm and 21 cm was constant during the period 1963–67 (refs 8–10); and observations at 13 cm showed no variations larger than 2% during a 6-month interval in 1971 (ref. 1).

Variations in the intensity of Jupiter's radio emission is relevant to the analysis of particle and field data returned from spacecraft such as Pioneer 10 and 11. If the intensity variations are caused by physical changes within the Jovian radiation belts, the environment measured by Pioneer 11 will probably be different from that measured by Pioneer 10.

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Shape of Mars

THE geometrical ellipticity of Mars differs considerably from the dynamical ellipticity obtained from the precession of the orbits of the satellites. Study¹ of the Martian centre of mass lead Schubert and Lingenfelter to comment that the location of the Hellas basin near the direction of the thinnest crust, as implied by the offset, might suggest that an impact produced the asymmetric crustal distribution. If such an impact did occur then it is likely to have also affected the shape of Mars. An order of magnitude estimate can therefore be made, from the shape of Mars, of the required size and velocity of the impacting body.

I take the value for the geometrical ellipticity e_s to be 0.0089 ± 0.0015 (ref. 2). For the dynamical ellipticity e , which is related to the second harmonic of the planets gravitational field, I take the value 0.005238 ± 0.00009 (ref. 3). Assume now that the rotation of Mars was greater at some past epoch for which the whole planet was in hydrostatic equilibrium. For such a case the ellipticity e_h can be defined as the fractional excess of the equatorial radius over the polar radius. The Darwin–Radau formula gives

$$e_h = \delta / \left[\frac{2}{5} + \frac{5}{2} \left(1 - \frac{3}{2} \frac{C}{Ma^2} \right) \right] \quad (1)$$

where δ is the ratio of the centrifugal to the gravitational acceleration at the equator, C is the moment of inertia about the axis of rotation, M is the mass and a is the mean radius of Mars. I now assume that as a first approximation the ratio C/Ma^2 and the ratio a^3/M may be treated as remaining constant. Then

$$e_h = k_1 \delta = k_2 \Omega^2 \quad (2)$$

where k_1 , k_2 are constants of proportionality and Ω is the angular rotation of Mars. The assumption of total hydrostatic equilibrium gives the relationship

$$e = e_s = e_h \quad (3)$$

Let Ω_1 be the angular rotation of Mars before impact and Ω_2 its present angular rotation; for simplicity consider the axis of

rotation as unchanged. I now assume that most of the planet is in hydrostatic equilibrium but that the surface has approximately the old ellipticity. Thus to obtain the value of Ω_1 which will make $e_h = 0.0089$, $\Omega_1 \approx 1.3\Omega_2$ (from equation (2)).

To obtain some idea of the size and velocity which an impacting body may have to have in order to achieve this reduction in the angular velocity I consider a simple two dimensional situation where a body of mass m impacts with a velocity v tangentially to the surface of Mars and along its equator. I assume that the direction of impact is such as to reduce the rotation of Mars.

In an impact of this size dispersion of material would be expected to result and in a more exact treatment one would have to integrate the angular momentum about the axis of rotation of Mars for a distribution of secondary impacting bodies as well as for the primary object. Instead I assume a non-elastic rigid body impact which makes our treatment an approximate one. If the moment of inertia of Mars (about its axis of rotation) is $0.376Ma^2$ then from conservation of angular momentum

$$\begin{aligned}mv &\approx 0.376Ma(\Omega_1 - \Omega_2) \\ &\approx 0.1Ma\Omega_2 \\ v &\approx 20(M/m)\end{aligned}\quad (1)$$

since $a\Omega_2 \approx 200 \text{ m s}^{-1}$.

The orbits of the asteroids are very varied; some have eccentricities of 0.8 or greater. Some asteroids have orbits which take them past the orbit of Jupiter. I now select an orbit which will give a large impacting speed and which is compatible with existing asteroid orbits to see whether this will give a reasonable mass for the impacting body. The orbit chosen is one which has an ellipticity of 0.8 and which comes close to the orbit of Jupiter. For simplicity take the orbit of Mars as a circle with unit radius. Take the elliptical orbit to be $r_{\max} = 3$ and $r_{\min} = \frac{1}{3}$ where $r_{\max}$ and $r_{\min}$ are the aphelion and perihelion distances. This gives a value of 0.8 for the ellipticity e , and $r_{\max}$ is just less than the mean radial distance of Jupiter.

If I now take the Sun as origin I have for the equations of the orbits of Mars and of the impacting body

$$x^2 + y^2 = 1, \quad (x-c)^2 + y^2/(1-e^2) = a^2$$

where $a = 5/3$, $e = 4/5$ and $c = ae = 4/3$. For such an orbit (on calculations made using standard two-dimensional orbit theory) the speed of the impacting body at the moment of impact is $\sim 30 \text{ km s}^{-1}$ at an angle of $\sim 19^\circ$ to the major axis of the elliptical orbit. The speed of Mars is $\sim 24 \text{ km s}^{-1}$ at an angle of $\sim 150^\circ$ to the major axis of the elliptical orbit. So the impact speed relative to Mars is $\sim 32 \text{ km s}^{-1}$. This value could be increased slightly if the acceleration due to gravitational attraction was to be taken into account. Substituting this value for v in equation (1) gives as the required mass of the impacting body the value $m \approx 0.7 \times 10^{-3}M$. If the impact is assumed to be tangential at latitude 45° (approximately that of the Hellas basin) then $m \approx 10^{-3}M$ but if it is at an angle of 45° to the vertical then $m \approx 1.4 \times 10^{-3}M$. These values for m are about the same as the mass of the largest asteroid, assuming that the densities of Mars and of the asteroid are the same.

These calculations were made on the assumption that the difference between the geometrical and dynamical ellipticities is due to collision only. If other factors such as convection⁴ play a role the required size of the asteroid and the value of the orbital eccentricity of the impacting body may even be smaller.

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CO₂ and HCN in sunspots

THE triatomic molecules CO₂ and HCN have now been reported to be present in cool stars^{1,2}, and H₂O had already been detected in the sunspot spectrum³⁻⁷. Dissociation equilibrium calculations for three recent sunspot models⁸⁻¹⁰ show that the molecules CO₂ and HCN are fairly abundant in sunspots. The results for the Zwaan model have already been published¹¹; we further find that the abundances of CO₂ and HCN are comparable for all three models. We have therefore calculated equivalent widths of selected lines of these molecules to ascertain if they might show up in the infrared region of the sunspot spectrum. The strongest fundamental bands of HCN and CO₂ and a weaker band of CO₂ were included in our calculations. The selected J values correspond closely to the expected maximum Local Thermodynamic Equilibrium (LTE) population under sunspot conditions.

The sunspot model selected for the equivalent width calculations is that of Stellmacher and Wiehr¹⁰. The procedure for calculating the equivalent widths has been outlined earlier¹². Two R (56) lines of CO₂ at wavenumbers 3,478.93 cm⁻¹ and 2,384.49 cm⁻¹, belonging respectively to the bands (10⁰1-00⁰) and (00⁰1-00⁰) of the Σ - Σ transitions, were selected. The equivalent width of the former line at the centre of the disk turned out to be 0.032 mÅ suggesting an absence of the (10⁰1-00⁰) band in the sunspot spectrum. Details of the centre-to-limb variation of equivalent width for the (00⁰1-00⁰) line are given in Table 1.

Table 1 Equivalent width of (00⁰1-00⁰) line

cos θ	1	0.7	0.5	0.3
Equivalent width (mÅ)	6.6	7.7	7.8	11.3

We are not aware of any observations of the (00⁰1-00⁰) band of CO₂ in the 4.3 μm region of the sunspot spectrum and therefore a verification of these predictions with observations has yet to be done.

The R (25) line of HCN at 3,380.84 cm⁻¹, belonging to the band (00⁰1-00⁰) of the Σ - Σ type transition, in the Stellmacher-Wiehr¹⁰ sunspot model has an equivalent width of 0.072 mÅ at the centre of the disk. The fact that the other fundamental bands at 712 cm⁻¹ and 2,089 cm⁻¹ have lesser integrated intensities¹³ leads us to suggest that the HCN fundamental bands may not show up in the infrared region of the sunspot spectrum.

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Re-interpretation of Devensian Till stratigraphy of eastern England

THE current reconstruction of the Devensian Till stratigraphy of Holderness¹ invokes three units—the Drab, Purple and Hesse Tills—with the Hesse overlapping both lower tills so as to be the only one present in the westernmost part of the area, on the Yorkshire Wolds. Because of the characteristic reddish brown colours (usually 5YR4/4 of the Munsell Soil Colour Charts, 1954) of the uppermost tills throughout eastern England, the upper till in Lincolnshire and the Hunstanton Till of north Norfolk are equated usually with the Hesse Till¹⁻⁵, although Straw⁶ correlated his two Marsh Tills in Lincolnshire with the Drab and Purple Tills of Holderness, and Bisat⁷ equated the Hesse Till of the Yorkshire Wolds with his Middle Drab division.

The results of a mineralogical study on these tills⁸ have shed new light on these stratigraphic correlations. In Yorkshire, the Drab and Purple Tills are found to be essentially uniform in both texture and mineralogy, but distinct from one another, whereas the Hesse Till undergoes a transition in these properties between south-eastern Holderness and the Wolds (Fig. 1a) may be drawn for various textural and mineralogical parameters, on the basis of which three zones can be distinguished (Fig. 1b). Zone C covers western and northern parts of Holderness and the Yorkshire Wolds. No mineralogical or textural distinctions could be drawn between Straw's⁶ Upper and Lower Marsh Tills, and both these and the Hunstanton Till resemble closely the Hesse Till of Zone C in Holderness. This till is also texturally and mineralogically similar to the Drab Till, on which it can be seen to lie in deep sections. The only significant differ-

ences are the colours of the two tills, and the almost complete absence of siderite and pyrite from the coarse silt (20–53 μm) and fine sand (53–250 μm) fractions of the Hesse Till. The Hesse Till of Zones A and B likewise resembles the Purple Till in all but these respects. The use of selected mineral ratios (Fig. 2) brings out most clearly the relationships between the various tills.

The coarse silt mineralogy of the Hesse Till of Zone C resembles that of loessial material that overlies the chalk on the Yorkshire and Lincolnshire Wolds and is also incorporated in a chalky head deposit beneath the Hesse Till⁹. Incorporation of loess in the till of western Holderness and the Wolds could thus be invoked to account for the change in composition of the Hesse Till. A large admixture of silt would however, be necessary but the Hesse Till of Zone C contains less silt than that of Zones A and B. Also, there are parallel changes in the fine sand mineralogy that could not be explained in this way, as loess contains very little fine sand.

The variability of the Hesse Till across Holderness may be related to the direction of ice movement, as the isopleths (Fig. 1a) are approximately parallel to Devensian ice fronts postulated by several authors¹⁰. Similar variations occur in Wisconsinian tills in Ohio and Pennsylvania and are ascribed to progressive incorporation of underlying bedrock of a mechanical and mineralogical composition different from that of the original till material¹¹. There, however, the changes were greatest in the lowest till, whereas in Holderness it is the uppermost till that is most variable. Thus, the 'contamination hypothesis' only seems tenable if the Hesse Till was the product of a separate ice advance. Catt and Penny¹ could find no evidence to support that

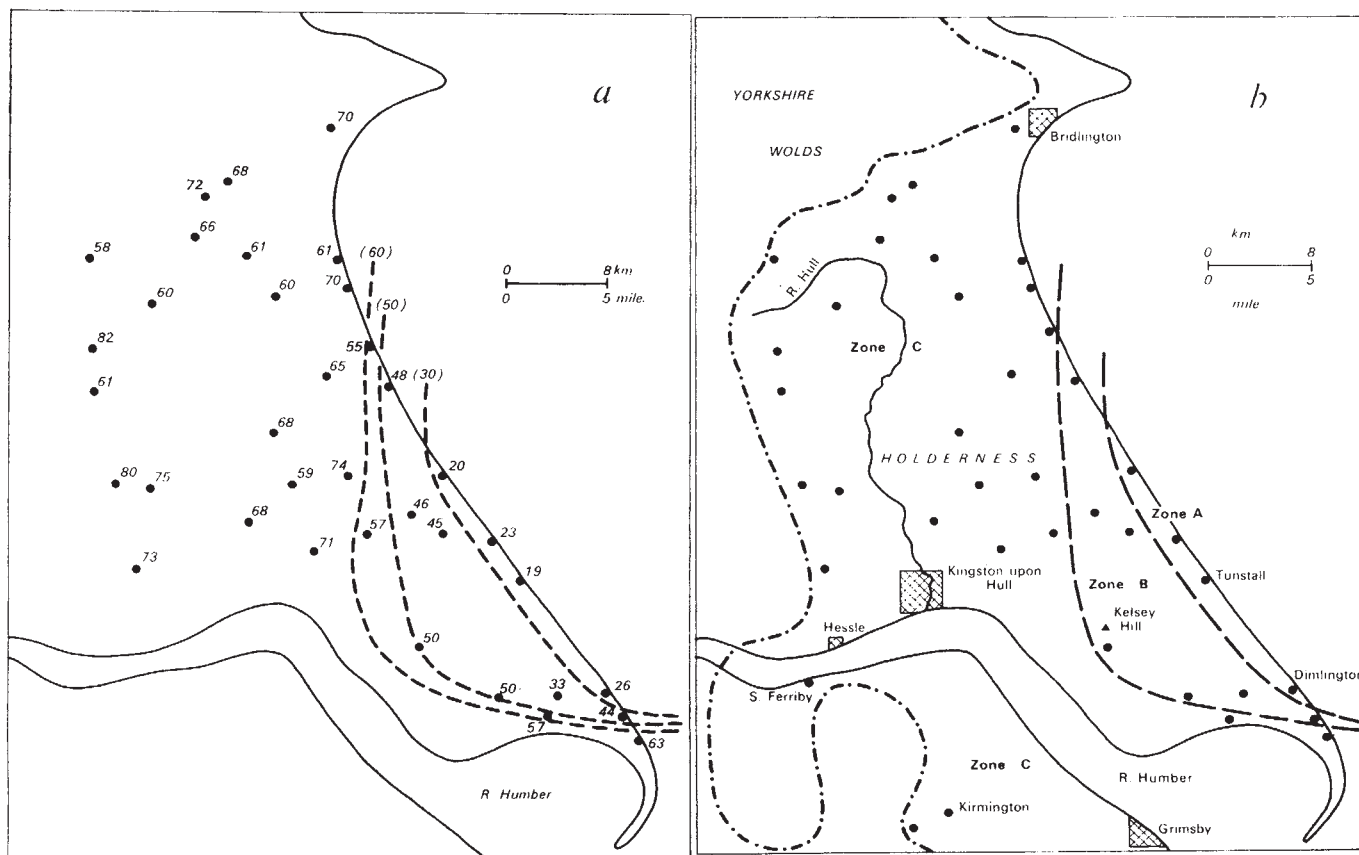


Fig. 1 a, Mineralogical variability of the Hesse Till, 53–250 μm fraction (amphibole/(amphibole+pyroxene) $\times 100$); b, zonation of the Hesse Till in Holderness: ●, sampling sites; ---, western limit of Devensian tills.

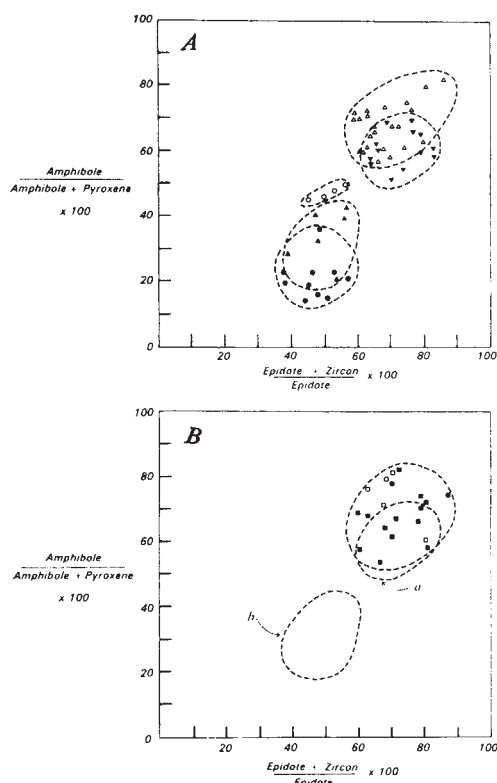


Fig. 2 Relationships between tills shown by mineral ratios. A: ●, Hesse Till, Zone A; ○, Hesse Till, Zone B; △, Hesse Till, Zone C; ▲, Purple Till; ▼, Drab Till. B: ■, Marsh Till; □, Hunstanton Till; a, Drab Till; b, Purple Till.

suggestion, and regarded the entire Drab–Purple–Hesse sequence as the product of a single, composite Devensian ice sheet.

Most of the variation in the Hesse Till seems to be related to the extent of the Purple Till, which is seen only in the cliffs of south-eastern Holderness and probably thins out westwards from the coast, as it is absent in the Hull Valley'. The Hesse Till of Zone A is always underlain by Purple Till, and is mineralogically and texturally similar to it, whereas that of Zone C overlies Drab Till or Chalk, and is mineralogically and texturally similar to the Drab Till. The Hesse Till of Zone B occupies an intermediate position,

geographically, mineralogically and texturally, and may coincide with the feather edge of the Purple Till sheet.

The exact junction between the Hesse Till and the underlying Drab Till is difficult to place, because the colour change often extends vertically downwards along fissures in the underlying grey (Drab) till, giving rise to reddish brown partings within it. Colour boundaries such as that indicate oxidative weathering, and siderite and pyrite, which are common in the Drab and Purple tills, but virtually absent in the Hesse, Marsh and Hunstanton tills, are both oxidised easily to hydrated ferric oxides, which would give the oxidised till a reddish brown colour. The grey Lower Marsh Till at South Ferriby (SE 996223) in Lincolnshire contains both siderite and pyrite, whereas the red till above it, which resembles the Hesse Till, lacks both minerals but is otherwise texturally and mineralogically identical to the grey till. Samples collected at metre intervals through the Hesse Till into the Purple Till at Dimlington (TA 398207) also proved to be mineralogically similar except for siderite and pyrite, both of which were present in samples close to and below the colour change taken as the boundary between the two tills. Further samples across the boundary between Purple and Drab tills at Dimlington High Land (TA 393215) and Aldbrough (TA 259395) showed that a mineralogical gradation occurs over a few metres close to this junction.

That leads to the conclusion that the Hesse Till, as currently defined, is in reality a composite unit. The reddish brown till of Zone C is the weathered upper part or feather edge of the Drab Till, and that of Zone B is probably the weathered remains of the thin westward extension of the Purple Till. That of Zone A is the weathered upper layer of the Purple Till. The name 'Hesse Till' is thus misleading stratigraphically; at the type locality it is, under the new interpretation, weathered Drab Till, whereas in south-eastern Holderness it is weathered Purple Till. Therefore, I propose that usage of the term be discontinued.

Straw⁶ subdivided the Marsh Tills of Lincolnshire into Upper and Lower units on the basis of their position either side of a 're-advance limit' of the last glaciation, which he recognised geomorphologically. I could detect no lithological differences between the two divisions; both tills were similar to the 'Hesse Till' of Zone C in Yorkshire. As this is now recognised to be weathered Drab Till, it seems reasonable to suggest that the Marsh Tills are also Drab or weathered Drab. A similar argument applies to the Hunstanton Till, which seems to be the oxidised, southernmost extension of the Drab Till. No till resembling either the

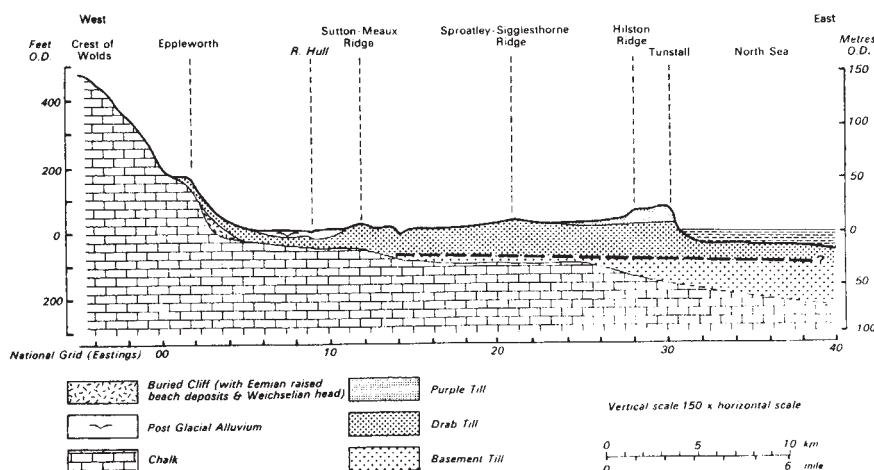


Fig. 3 East-west section across Holderness.

Purple or the 'Hessle' of Zone A occurs in Lincolnshire or northern Norfolk.

Many workers^{1,4-6,10} have cited the fresh, relatively undissected glacial morphology of Holderness, eastern Lincolnshire, and the coastal strip of northern Norfolk, in arguing for a Devensian age for the tills. This suggests the present distribution of the Drab and Purple Tills is depositional, and not primarily a result of subsequent erosion. The stratigraphic relationship of the Devensian Tills of eastern England is therefore one of offlap (Fig. 3) and not of overlap¹.

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Copper in surface waters south of New Zealand

THE sampling of the transition metals, including copper, in the ocean has so far produced unsatisfactory results. Reported measurements show quite a large scatter within a given suite of data and significant differences between investigators¹⁻³. Published profiles in general look quite unlike any of the 'accepted' distributions for other dissolved species outlined later nor are there any close similarities between the transition elements themselves. Either they have a unique geochemistry or the data are to some degree invalid. For copper, contamination during sampling has been suggested⁴ as one source of error, and we

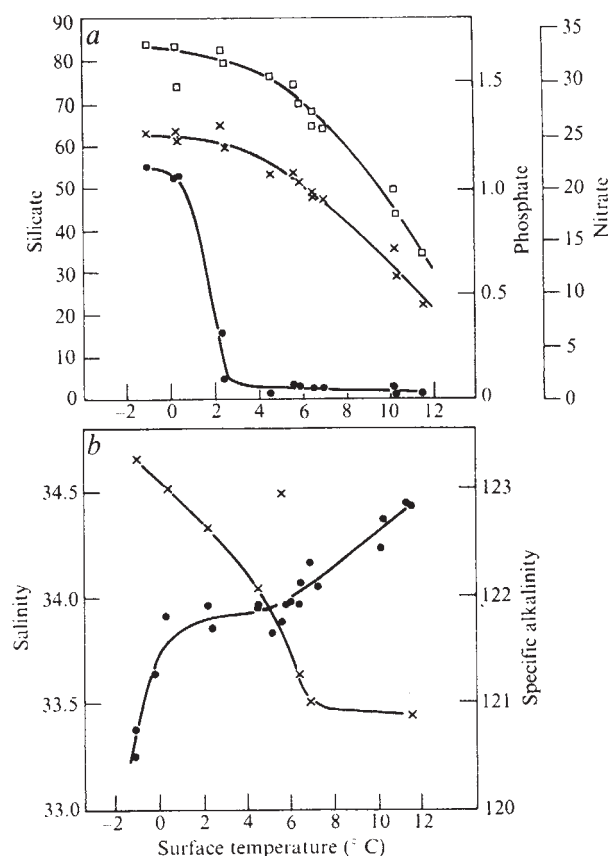


Fig. 1 *a*, Plots of surface values for phosphate, nitrate and silicate for all stations on GEOSECS Leg G against surface temperature. Units are μmol kg⁻¹. □, Phosphate; ×, nitrate; ●, silicate. *b*, Plots of specific alkalinity and salinity against surface temperature. For specific alkalinity (alkalinity/chlorinity) the unit is μeq per g chloride. ×, specific alkalinity; ●, salinity.

have tried to overcome this by sampling surface waters in regions of marked horizontal gradients in chemical properties.

Present understanding of the controls on the distribution of dissolved species in the ocean has been derived largely from inferences based on the form of the distributions themselves. Five types are presently recognised:

- (1) constant relative composition (Na, K, Mg, Cl, SO₄²⁻) as a result of very low reactivity in the ocean environment;
- (2) surface depletion and deep water enrichment (P, N, C, Sr,

Table 1 Preliminary station data for surface waters on Leg G of the Pacific GEOSECS expedition

Station	Location	T (°C)	S (‰)	PO ₄ (μmol kg ⁻¹)	NO ₃ (μmol kg ⁻¹)	Si (μmol kg ⁻¹)	A _{sp} (μeq g ⁻¹)	Cu (nmol kg ⁻¹)
280	56°1'S, 170°3'E	6.45	34.061	1.30	19.2	2.9	121.3	2.27
282	57°35'S, 169°36'E	5.58	33.880	1.49	21.5	3.4	123.0	2.57
				1.55*	22.4	0.9		
284	59°31'S, 170°0'E	5.85	33.957	1.41	20.6	3.2	—	1.80
285	61°29'S, 169°58'E	2.24	33.963	1.65	25.9	15.9	122.7	2.52
				1.67*	26.6	15.6		
286	66°5'S, 173°40'E	0.30	33.907	1.48	24.5	52.7	123.0	3.05
				1.55*	25.2	53.8		
287	69°5'S, 173°30'W	-1.07	33.377	1.68	25.2	54.9	123.3	3.25
				1.59*	25.9	56.6		
290	58°0'S, 174°0'W	4.53	33.958	1.53	21.4	1.4	122.1	1.87
				1.52*	22.0	3.7		
292	54°5'S, 176°58'W	10.25	34.358	0.88	11.5	1.4	—	0.98
293	52°40'S, 178°5'W	11.47	34.426	0.69	9.0	1.8	120.9	1.33
294	50°38'S, 179°59'W	10.16	34.230	0.99	14.2	3.0	—	1.28

* Where the surface sample was collected more than 2 h before or after the shallow hydro cast, nutrients were also determined on the trace element water sample on the ship. The values are given below the station values.

IO_3^- , Si, Ca, alkalinity, Ba, ^{226}Ra)^{5,6} caused by incorporation in the biogeochemical cycle (dissolved oxygen is a special 'inverse' case involved in the same cycle);

- (3) mid-depth and near-bottom depletion (^{210}Pb , ^{210}Po) caused by adsorption on suspended particles;
- (4) pronounced mid-depth maximum (^3He) caused by injection at spreading ridge crests;
- (5) near-bottom enrichment (^{222}Rn , ^{226}Ra , ^{228}Ra) caused by diffusion of radiogenic isotopes out of sediments.

To date no categorisation has been made for any of the transition metals. Patterson⁴ has suggested that in spite of increasingly stringent precautions taken by trace element geochemists, a major unsolved problem is contamination during sampling. This problem can be most easily circumvented by restricting sampling to surface waters; however, the major criterion for validity of data is that it be oceanographically consistent. To apply this constraint requires sampling in regions of marked horizontal gradients in chemical properties. Such an opportunity arose on Leg G of the Pacific GEOSECS expedition, which made two sections south of New Zealand across the Circumpolar Current to $\sim 75^\circ\text{S}$. In this region major upwelling of deep water takes place, intensifying southwards. Plots of cruise data against surface temperature (Fig. 1) or latitude show

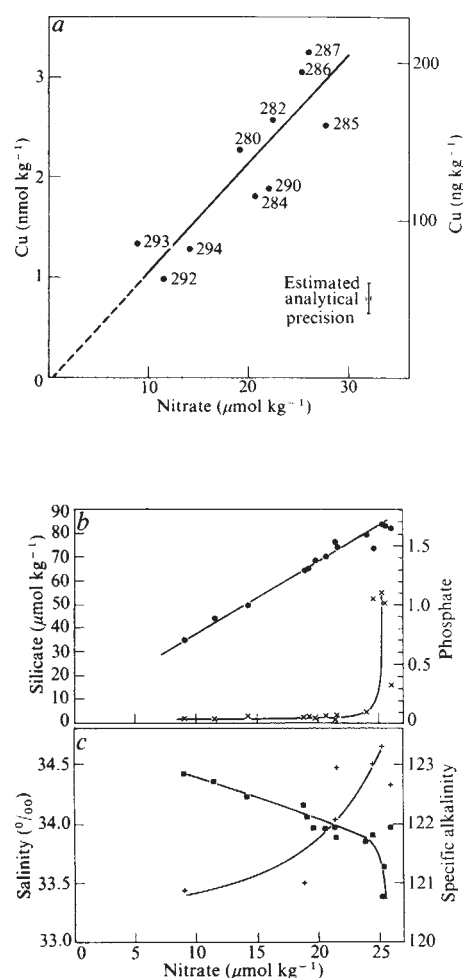


Fig. 2 a, Plot of the copper data against nitrate: station numbers are given adjacent to the points. b, Plots of surface phosphate and silicate against nitrate for all stations on Leg G. ●, Phosphate; ×, silicate. c, Plots of specific alkalinity and salinity against nitrate. ■, salinity; +, specific alkalinity.

systematic increases for phosphate, nitrate and specific alkalinity and a step-like distribution for silicate.

Samples were collected on station from the bow of the ship. The sampler was a 4-l polyethylene jerry jug which had been leached with 0.1 N hydrochloric acid for 24 h, rinsed four times with very pure distilled water, and stored in a polyethylene bag. The jug was supported in a harness of polypropylene line. A metal weight encased in plastic was suspended about 6 m below this with similar line. First, the capped jug was lowered into the water and the exterior rinsed. It was then retrieved, the cap removed, resubmerged, and allowed to fill. The jug filled at a depth of at least 3 m. On recovery the jug was capped and within 5 min acidified with about 20 ml of 6 N hydrochloric acid which had been redistilled from a Vycor still. The bottles were stored in sealed polybags until analysis 2 months later.

Copper was preconcentrated from the samples by coprecipitation with cobalt pyrrolidine dithiocarbamate. The precipitate was redissolved in a small volume of methylethyl ketone with 5% 0.1 N HNO_3 . Copper was then determined by flame atomic absorption: the estimated precision of the analysis is ± 0.15 nmol kg⁻¹ and the blank is 0.45 nmol kg⁻¹.

The results of the analysis are given in Table 1 together with the relevant hydrographic and station data. The copper concentrations are all near the low end of the range of recently reported values^{1,2} (1–32 nmol kg⁻¹) and exhibit variations of almost a factor of three that are correlated with surface temperature. In Fig. 2 the data are plotted against nitrate. This is the most precisely determined tracer of upwelling, the analytical precision being about 0.6% (for phosphate 0.7%). Also shown are similar plots for phosphate, silicate, salinity and specific alkalinity (A_{sp}). It is apparent from this and from Fig. 1 that the distribution of silica is quite distinct and that the copper–nitrate correlation resembles closely that for phosphate and alkalinity. The inverse relation for salinity reflects the precipitation patterns in the region: the existence of the Antarctic Circumpolar Front, at $\approx 4^\circ\text{C}$ precludes a simple mixing relationship.

The linear copper–nitrate trend has a least-squares standard error of 0.3 nmol kg⁻¹ and the line passes through the origin (-0.07 nmol kg⁻¹ at zero nitrate). The correlation coefficient is 0.88 and the molar ratio Cu:N is 1.09×10^{-4} giving a 'Redfield ratio' Cu:P of 1.7×10^{-3} . The recently reported average in plankton ash⁷ is 6.6×10^{-3} . Although the covariance of copper with the nutrients phosphate and nitrate is plausible, given the biochemical importance of copper⁸, it is not uniquely established from these data.

The scatter in the alkalinity data makes it difficult to relate to the other nutrients: a covariance of copper and alkalinity would, however, require that biogenic carbonates contain approximately 60 p.p.m. Cu. Arrhenius⁹ reported 25 p.p.m. in foram ash with 15 p.p.m. being associated with the carbonate fraction. Aragonitic corals generally show values less than 10 p.p.m. (ref. 10). *Acantharia radiolaria* which precipitate shells of celestite (SrSO_4) have been reported as containing 750 p.p.m. Cu (ref. 9). Accepting the general correlation of strontium with phosphate in the water column the average Sr:P ratio of 0.67 would require 840 p.p.m. Cu in the strontium carrier phase. Extrapolation of these hypothetical copper–carbonate and copper–strontium relationships gives zero copper at a specific alkalinity value of 119 and a strontium concentration of 87 μmol kg⁻¹, that is, copper is exhausted long before the major carrier components: this is highly unusual behaviour for a solid solution.

In conclusion, it is definitely established that relative to lower latitudes, copper is enriched in the surface waters of the Antarctic upwelling areas, and, by extension, in the deep waters of the ocean. If the correlation with nitrate is valid as described here, then copper can be classified as a limiting nutrient. The levels in the surface water in low and mid-latitudes should be very close to zero (less than 0.1 nmol kg⁻¹) and the maximum values in the deep Pacific should not exceed 5 nmol kg⁻¹.

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Drag reduction by collapsed and extended polyelectrolytes

I REPORT experiments on drag reduction by saline solutions of a partially hydrolysed polyacrylamide (PAMH) showing the effect of macromolecule conformation. The extreme collapsed and extended PAMH conformations also simulate the deportment of linear random-coiling macromolecules in solution, and of high aspect ratio fibres in suspension; and they indicate a possible relationship between the mechanisms of drag reduction produced by these additives.

A commercial PAMH polymer, B1110 (Betz Laboratories), $M = 15 \times 10^6$ was used. This has an intrinsic viscosity, $[\eta] = (2,300, 2,550, 6,000, 16,000, 52,000) \text{ cm}^3 \text{ g}^{-1}$ in (1.0, 0.1, 0.01, 0.001, 0.0) M NaCl solutions respectively. The radius of gyration of B1110 in 1.0 M NaCl was 315 nm; in dilute distilled water solution B1110 is expected to be greatly extended, to an end-to-end distance of the order of its contour length, $\sim 70 \mu\text{m}$.

Friction results for collapsed and extended conformations are shown in Fig. 1, using Prandtl-Karman coordinates with abscissae based on solvent viscosity. The behaviour of solutions of collapsed polyelectrolyte, Fig. 1a, is identical with that established¹ for random-coiling macromolecules. Solutions of 1 and 10 parts per million (by weight) of B1110 follow curve (1) for $Re_s f^{1/2} < 200$, transit to curve (2), exhibit an onset of drag reduction at $Re_s f^{1/2*} \sim 400$ and follow straight lines for $Re_s f^{1/2} > Re_s f^{1/2*}$ with slopes exceeding that appropriate for a Newtonian fluid. B1110, 100 p.p.m., follows curve (1), $Re_s f^{1/2} < 250$, and curve (3), $Re_s f^{1/2} > 350$. Among solutions of extended polyelectrolyte, Fig. 1b, 10 p.p.m. B1110 in distilled water follows curve (1) for $50 < Re_s f^{1/2} < 200$; for $200 < Re_s f^{1/2} < 400$ it seems to transit to the maximum drag reduction asymptote, but at $Re_s f^{1/2} \sim 400$ it breaks away into the polymeric regime, and then for $500 < Re_s f^{1/2} < 2,000$ it describes a straight line, $(S_p, I_p) = (5.0, 3.1)$, roughly parallel to but lying above the Prandtl-Karman law. Qualitatively similar behaviour occurs at 3 and 30 p.p.m. Solutions of 100 p.p.m. show marked shear thinning in laminar flow (power law index 0.60 ± 0.01 for $40 < Re_s < 4,000$) and for $Re_s f^{1/2} > 400$ seem to transit to the maximum drag reduction asymptote, the data points lying nearly parallel to curve (3) for $700 < Re_s f^{1/2} < 1,500$ with horizontal separation corresponding to $\eta_{rel} = 1.5$. So the

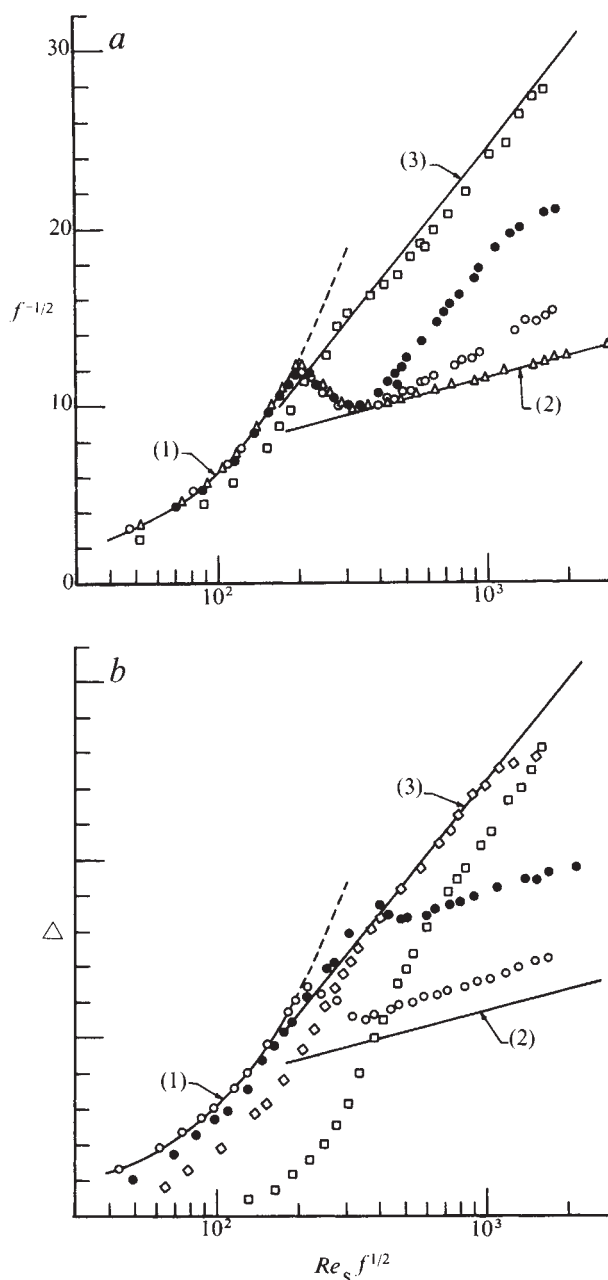


Fig. 1 Drag reduction by solution of a, collapsed polyelectrolyte; b, extended polyelectrolyte. Pipe internal diameter 0.945 cm, length 189 cm; polymer B1110, temperature $21 \pm 2^\circ \text{C}$. The solid lines represent (1) Poiseuille's law (denoted L), (2) the Prandtl-Karman law (N), and (3) the maximum drag reduction asymptote¹ (M). In the region between (2) and (3), called (4) the polymeric regime (P), polymer solutions can be seen to follow approximately linear relationships defined by their slope and intercept (S_p, I_p) . a: Δ , Distilled water (DW); $\circ$, 1.0 p.p.m. in 0.1 M NaCl; $\bullet$, 10.0 p.p.m. in 1.0 M NaCl; $\square$, 100.0 p.p.m. in 0.1 M NaCl. b: $\circ$, 3.0 p.p.m.; $\bullet$, 10.0 p.p.m.; $\star$, 30.0 p.p.m.; $\square$, 100.0 p.p.m. All in DW.

maximum drag reduction possible with extended polyelectrolytes seems to be limited by the usual asymptote, curve (3); with increasing $Re_s f^{1/2}$, the typical solution follows a path sequence LMP which contains a characteristic 'retro-onset' point $Re_s f^{1/2*}$ at which the polymeric regime segment departs from curve (3). Both slope and intercept of the polymeric regime segment increase with increasing polyelectrolyte concentration but all slopes are characteristically close to Newtonian. The LMP trajectory contrasts strikingly with the LNP trajectory typical of random-coiling polymer solutions.

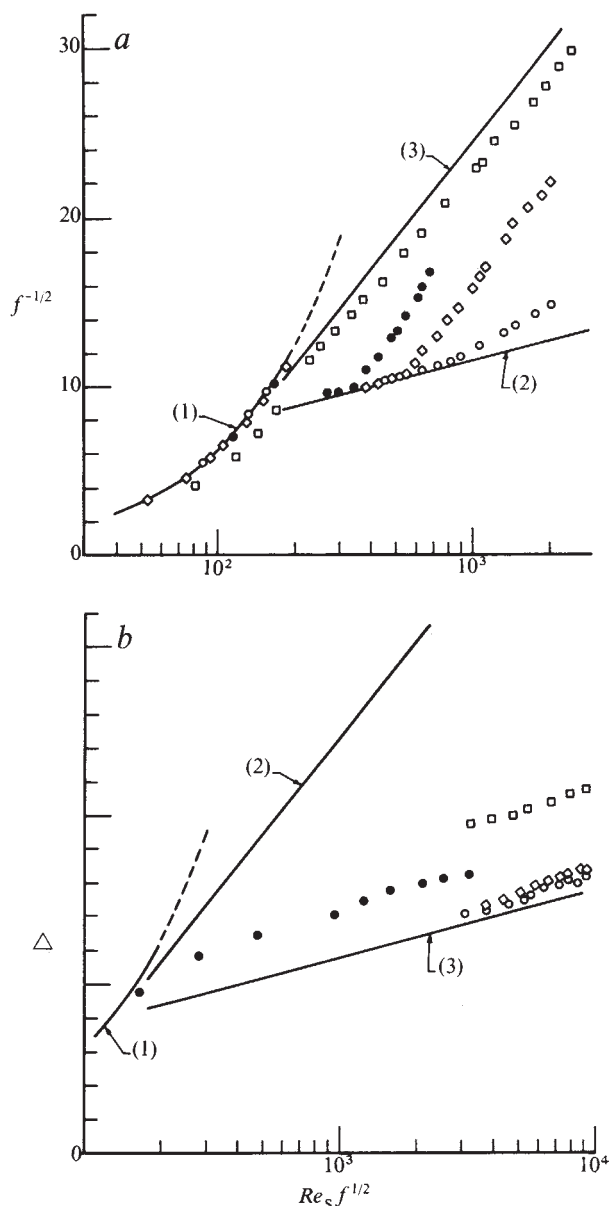


Fig. 2 Drag reduction by *a*, random-coiling polymer solutions; *b*, fibre suspensions⁴⁻⁶. *a*: ○, 10.0 p.p.m., W205; ★, 100.0 p.p.m. W205; ●, 10.0 p.p.m., FRA; □, 110.0 p.p.m., FRA. All DW solutions, all pipe 0.945 cm internal diameter except □, i.d. 0.846 cm. *b*: ○, 10⁴ p.p.m., nylon; ●, milling yellow crystals; ★, 800.0 p.p.m. asbestos; □, 2,500.0 p.p.m., asbestos. ○, i.d. 4.98 cm; ●, i.d. 7.79 cm; ★, and □, i.d. 5.08 cm.

Study of 10 p.p.m. B1110 in 1.0, 0.01, 0.001 and 0.0 M NaCl revealed a continuous variation in flow behaviour between the extremes shown in Fig. 1. In terms of drag reduction, measured by fractional flow enhancement $S_F = (-1 + (f_p^{-1/2}/f_s^{-1/2}) Re_s f^{1/2})$, relative efficiencies were $S_F(1.0 \text{ M}) < S_F(0.01 \text{ M}) < S_F(0.001 \text{ M}) > S_F(0.0 \text{ M})$, so a partially extended conformation was 'optimal' for $300 < Re_s f^{1/2} < 2,000$. Relative efficiencies of different conformations were, however, decidedly flow dependent; in Fig. 1 the 1.0 M and 0.0 M NaCl solution trajectories intersect making $S_F(1.0 \text{ M})$ less than, equal to and greater than $S_F(0.0 \text{ M})$ for $Re_s f^{1/2}$ less than, equal to and greater than 1,000 respectively. Possibly, therefore, the apparent conflict between the data of Parker² and of Hand³ may not be serious.

The present results can be related to drag reduction by random-coiling polymer solutions and by fibre suspensions using Fig. 2. Part *a* shows flow data for two polyethyleneoxide (PEO) polymers, FRA, $M = 8 \times 10^6$ and W205, $M = 1.3 \times 10^6$. PEO macromolecules possess a skeletal structure similar to

that of PAMH, and the FRA chosen has roughly the same number of skeletal links, $N \sim 0.5 \times 10^6$, as B1110. There is close correspondence between the 10 and 100 p.p.m. B1110 data in Fig. 1*a* and the 10 and 110 p.p.m. FRA data in Fig. 2*a*. Figure 2*b* illustrates drag reduction by fibre suspensions using data reported for suspensions of a nylon fibre⁴, milling yellow crystals⁵ and two chrysotile asbestos fibres⁶. The fibre suspensions all produce approximately straight lines which lie in the region between curves (2) and (3), possess slopes only slightly greater than the Prandtl-Karman, 4.0, and yield essentially constant drag reduction, independent of $Re_s f^{1/2}$. The milling yellow suspension yields $(S_p, I_p) = (4.5, 0.8)$ and $S_F \sim 0.23$ (constant) for $300 < Re_s f^{1/2} < 3,000$. The gross flow behaviour (Fig. 2*b*) which seems to be characteristic of fibre suspensions, is analogous to that exhibited by extended polyelectrolyte solutions (Fig. 1*b*) in the polymeric regime.

Results obtained with collapsed and extended polyelectrolyte possibly represent two extremes of drag reduction behaviour, one (designated Type A) which is characteristic of flexible, random-coiling, deformable additives and the other, (Type B) which is characteristic of relatively rigid, elongated, undeformable additives. Idealised, a family of Type A solutions yield polymeric regime segments fanning outwards from a common onset point on the Prandtl-Karman line with slopes increasing with increasing additive concentration and drag reduction increasing with increasing $Re_s f^{1/2}$. A family of Type B solutions yield polymeric regime segments coming off the maximum drag reduction asymptote at characteristic retro-onset points and lying roughly parallel to but displaced upwards from the Prandtl-Karman line with drag reduction essentially independent of $Re_s f^{1/2}$ but increasing with increasing additive concentration.

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Novel reactions of hydrocarbon complexes of metal-substituted sheet silicates: thermal dimerisation of *trans*-stilbene

TRANSITION metal, ion-exchanged montmorillonites form complexes^{1,2}, often of an interlamellar kind (intercalates), with a range of aromatic molecules, including benzene, toluene and the xylenes, anisole, and other species such as *trans*-stilbene and indene³⁻⁵. The aromatic molecules can form two main types of complex with the interlayer transition-metal ions, for example Cu(II). In the first type there is thought to be an edge- π -bonded copper arene moiety, similar to the bonding situation which exists in $C_6H_6CuAlCl_4$ (ref. 6), and in the second instance it is considered to be a type of bond which causes associated distortion of the aromatic ring and some localisation of the circumferential double bonds¹. Although detailed crystallographic analyses of the organic intercalates of sheet silicates has only recently commenced (ref. 7, and J. M. Adams *et al.*, unpublished) and in spite of the paucity of information relating to the precise nature of the bonding of organic entities to encaged metal ions, it is clear²⁻⁴ that these organic complexes display a rich range of unusual reactions, some new examples of which we report here.

Complexes of benzene, toluene, the xylenes and *trans*-stilbene were formed with dehydrated Cu(II)-exchanged montmorillonite by exposing the latter to the vapour of each

Table 1 Relative intensities of the mass spectral peaks of the thermal decomposition products.

Toluene complex 150° C		<i>p</i> -xylene complex 150° C		<i>trans</i> -stilbene complex 200° C	
<i>m/e</i>	Intensity	<i>m/e</i>	Intensity	<i>m/e</i>	Intensity
272	17	315	12	450	2
270	18	314	26	449	3
257	18	300	11	372	1
255	20	299	38	370	2
195	10	249	24	360	92
182	13	210	42	346	8
181	19	209	35	344	5
167	16	195	59	280	18
165	23	193	42	279	12
105	25	119	41	270	65
92	62	118	56	269	73
91	106	106	68	252	18
		105	100	239	8
				203	9
				202	13
				191	69
				180	77
				179	76
				178	100

respective aromatic material. Excess *trans*-stilbene was removed by washing with chloroform, and surplus solvent was removed by suction. On heating the complexes *in situ* in an AEI MS30 mass spectrometer, in high vacua, between 50 and 250° C, high molecular weight products appeared.

With the benzene complex of Cu(II) montmorillonite, which is known to involve the second type of linkage between the metal and the aromatic ring, only a negligible proportion of material possessing a molecular weight approximately two or three times that of benzene was produced, whereas with the toluene complex, which has the first type of linkage, appreciable quantities of molecules of mass number 272 and 182 were formed (Table 1). These mass numbers correspond to three toluene units less four hydrogen atoms and to two toluenes less two hydrogens, respectively. Complexes of all three xylenes, which have similar copper-arene linkages to the toluene complex, behaved very similarly and yielded mass numbers of 314 and 210, again signifying the loss of hydrogens when three and two units, respectively, of xylene condense. Blank mass-spectrometric experiments carried out with sodium-ion-exchanged montmorillonite revealed that, essentially, no hydrocarbon of higher molecular weight was formed when traces of physically adsorbed alkyl benzene were heated in the absence of the transition-metal ion.

With *trans*-stilbene, the results were strikingly different in that significant quantities of material possessing a mass number of 360, which corresponds to the dimer, were obtained. (Some ions with *m/e* equal to 370–450 were observed in low abundance, so some trimer or even higher order species must have been formed, although the molecular ion(s) corresponding to such species were not observed.)

Although much remains to be learned about the mechanisms of these thermally induced reactions the obvious major difference in the nature of the products obtained suggests that different reaction pathways are followed depending on whether the organic molecules contain aromatic rings or aromatic rings together with ethylenic double bonds. The implication, which needs to be tested, is that the central olefinic double bond in *trans*-stilbene is bonded to the Cu ion and that, as a result of the proximity and the electronic perturbation of these olefinic double bonds of the two (or more) *trans*-stilbene molecules, dimerisation is facilitated. Though it is unnecessary to postulate that the dimerisation is concerted, it is of interest to note that the thermal production of tetraphenyl-cyclobutane from *trans*-stilbene monomers is, by orbital symmetry, forbidden and that the photochemical dimerisation, though allowed, is not easily achieved either in the solid state^{8,9} or when weakly physically adsorbed¹⁰.

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Systematic position of *Plesiadapis*

THE systematic position of the Palaeocene mammal *Plesiadapis* has been a subject of discussion for almost a century. This is not surprising considering the meagre evidence on which it was first associated with lemuroid primates. A review of the evidence now available indicates that the affinities of *Plesiadapis* and its relatives are with early tarsier-like primates, and not with archaic lemurs.

Gervais¹ described the genus *Plesiadapis* after he² and Delfortrie³ had recognised *Adapis* as a primate related to living lemurs. Gervais' species *Plesiadapis tricuspidens* was originally based on two specimens collected by Lemoine from the Palaeocene of France—a mandible fragment and an isolated incisor—neither of which show any significant resemblance to *Adapis*. The relationship between *Plesiadapis* and *Adapis* advocated by Gervais was apparently based on several Eocene specimens first described by Lemoine as *Plesiadapis*⁴, but subsequently transferred to the new, genus *Protoadapis*⁵ (which is closely related to *Adapis*).

Once given a name compounded from *Adapis*, no matter how poorly justified that actually was, it is natural that *Plesiadapis* was subsequently compared most closely with lemur-like primates. Simpson⁶ emphasised the detailed resemblance of the molar pattern of *Plesiadapis* to that of the early adapid *Pelycodus*, and classified the Plesiadapidae as a family within the Lemuroidea⁷. The presence of a postprotocingulum ('*Nannopithecus*-fold') on the upper molars of *Plesiadapis*, *Pelycodus*, and early Eocene tarsioid primates is an important derived characteristic shared by early primates⁸ but, considering the important differences now known in the anterior dentition of species of *Plesiadapis* and *Pelycodus*, it appears that Simpson overestimated the significance of their molar resemblances. *Plesiadapis* also differs significantly in middle ear structure from early lemuroid primates.

In a group of mammals with a reasonably good fossil record, a biostratigraphic approach to phylogeny⁹ offers the best evidence for working out the true relationships between species. Virtually all specimens assigned to the family Plesiadapidae have been studied again in a carefully documented biostratigraphic context¹⁰. Five plesiadapid lineages are known, at least two of which were common to both Europe and North America. Particularly interesting here is the major lineage leading from *Pronothodectes matthewi* of the middle Palaeocene (Torrejonian) to *Platychoerops richardsonii* of the early Eocene (Cuisian).

Plesiadapis tricuspidens is a late member of this central lineage. As the earliest plesiadapid and the common ancestor of all the later species known, *Pronothodectes* is the primitive form

which should be compared with members of other families in determining the relationships of Plesiadapidae.

Pronothodectes matthewi is similar in dental conformation to early species of Carpolestidae, Paromomyidae, and Microsyopidae (Paromomyidae is limited here to forms having tricuspid plesiadapid- or carpolestid-like upper incisors, including *Paromomys*, *Phenacolemur*, and possibly *Saxonella*; whereas the Microsyopidae, including *Plesiolestes*¹¹, *Palaeochthon*, *Palenochtha*, *Berruvius*, *Navajovius*, have simpler bicuspid upper incisors¹⁰.) All have a basic dental formula of

$$\begin{array}{cccc} 2 & 1 & 3 & 3 \\ 2 & 1 & 3 & 3 \end{array}$$

and all have enlarged, procumbent, pointed, lower central incisors. Early Tarsiidae and Omomyidae have the same basic dental formula and very similar lower incisors, with one possible exception: some specimens of *Teilhardina* apparently retained four premolars, although descriptions of the dental morphology of this genus are conflicting and deserve additional study. The lower dental formula of microchoerines is sometimes cited as 1.1.4.3 (ref. 12), but interstitial wear on the medial side of the enlarged anterior tooth shows it to be the central incisor, and the 'alveolus' in front of this tooth is an anterior mental foramen¹⁰.

Figure 1 shows that the mandibular and dental conformation of Palaeocene primates of the infraorder Plesiadapiformes is very much like that of Eocene Tarsiiformes (Plesiadapiformes Simons, 1972¹³ includes the same four families as Paromomyiformes Szalay, 1973¹⁴ and the former name is used here). The morphology of the anterior teeth differs fundamentally from that seen in the earliest lemuriform primate *Pelycodus* and its descendants. The lower central incisor (I_1) of *Pelycodus* is slightly smaller than I_2 and both are considerably smaller than the canine. Furthermore, the incisors of adapids differ in being vertically implanted and in having spatulate rather than pointed crowns.

The auditory bulla and middle ear are exceptionally well preserved in a skull of *Plesiadapis tricuspidens* recently collected by M. Pellouin of Reims from the Palaeocene locality of Berru in France. The auditory bulla in this skull was completely ossified, with no trace of a separate entotympanic element (though an entotympanic centre may have been present during ossification). Russell¹⁵ has described the extended external auditory meatus and fusion of the tympanic anulus into the lateral wall of the auditory bulla in another skull of *Plesiadapis tricuspidens*; the skull illustrated in Fig. 2 shows these characters even more clearly. An extended external auditory meatus and a tympanic anulus fused into the wall of the bulla characterise all Tarsiiformes but not primates of the infraorder Lemuriformes or

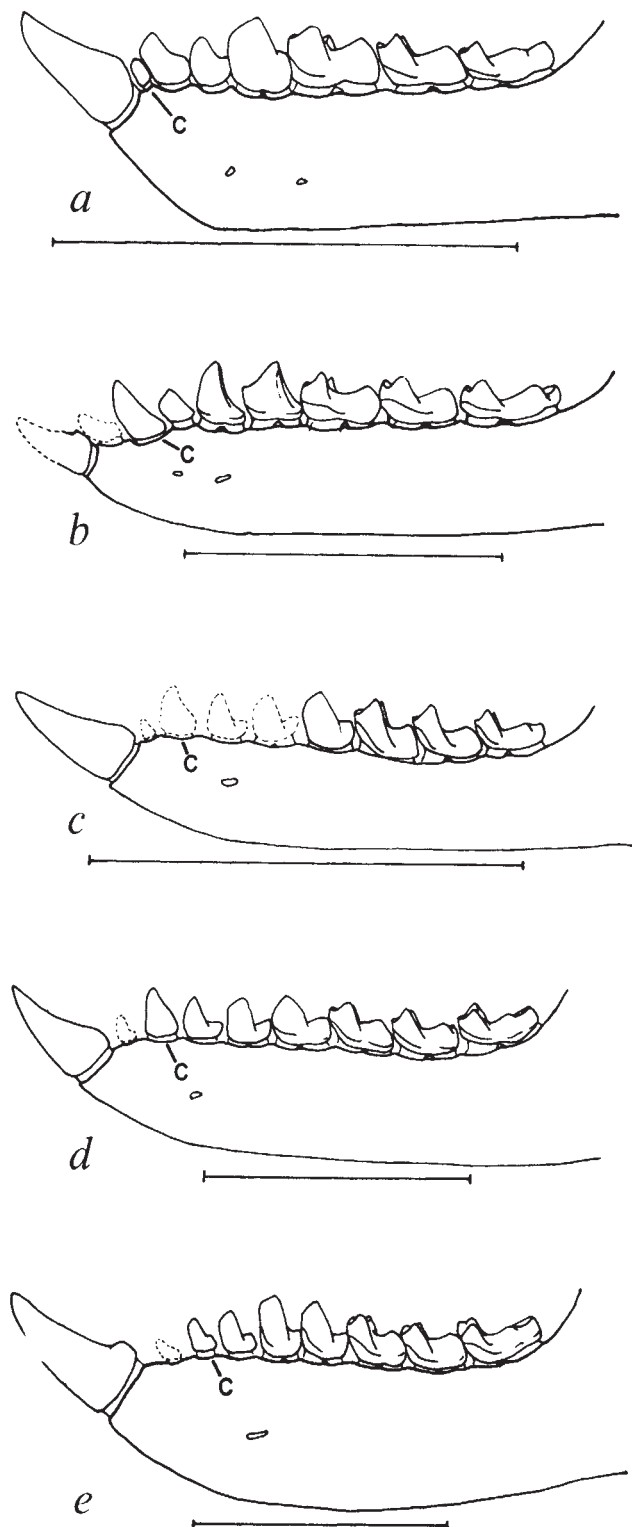


Fig. 1 Comparison of the lower dentition of representative Eocene Tarsiiformes (*Nannopithecus* (a), *Omomys* (b)) with that of middle Paleocene Plesiadapiformes (*Palenochtha* (c), *Plesiolestes* (d)), and the primitive plesiadapid *Pronothodectes* (e). Note enlargement of the central incisor distinguishing these forms from lemuriform and anthropoid primates. c, Lower canine. All figures in lateral view and brought to same size for comparison, bar represents 1 cm. Specimens are a, Halle IL-8, Geiseltalmuseum in Halle; b, AMNH 12600, YPM 13219, 16287, American Museum of Natural History, New York; c, PU 14786, 19461; d, PU 14149, 17427, Princeton University Museum, New Jersey; e, USNM 9332, National Museum of Natural History, Washington, AMNH 35462.

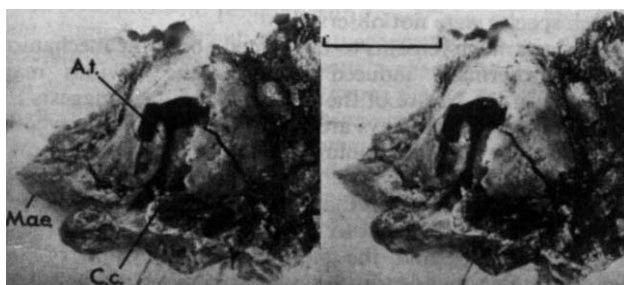


Fig. 2 Right auditory bulla of Pellouin skull of *Plesiadapis tricuspidens* in ventral view (stereophotograph). Note *Necrolemur*-like¹² struts anchoring the tympanic anulus to the lateral wall of the bulla. Fragments were removed from the ventral wall of the bulla to facilitate cleaning. A.t., Anulus tympanicus; C.c., Canalis caroticus; M.a.e., Meatus acusticus externus. This skull of *Plesiadapis* includes an almost complete maxillary dentition, on which its identification is based. Bar represents 1 cm.

primitive Anthropoidea. The ear region of the skull of *Plesiadapis* (and *Phenacolemur*¹⁶) thus furnishes additional evidence linking plesiadapiform primates to Eocene *Necrolemur*, Oligocene *Rooneyia* and living *Tarsius*, and to the origin of Tarsiiformes.

In both dental conformation and middle ear morphology, generally recognised as the two character complexes of greatest systematic importance among early primates, plesiadapiformes are very similar to early Tarsiiformes and differ greatly from early Lemuriformes. Evidence is presented elsewhere suggesting that Anthropoidea are derived from lemuriform rather than tarsiiform ancestors¹⁷. Thus there seems, from the fossil evidence, to be a basic dichotomy within the primates separating the infraorders Plesiadapiformes and Tarsiiformes on one hand from the infraorders Lemuriformes and Anthropoidea on the other^{10,18}. The earliest lemuriform primates appear abruptly in the fossil record, suggesting that they migrated to Europe and North America at the beginning of the Eocene (as did the earliest rodents and several other important groups). *Purgatorius*, from the Early Palaeocene¹⁹, is the only form known which could possibly be the last common ancestor of all later primates.

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Collective orientation in night-flying insects

SEVERAL investigations have been made using microwave radar techniques to study individual insects in free flight^{1–5}. One of the most surprising claims to result from these studies is that insects flying at night sometimes adopt a common orientation, usually downwind^{1,2,4}. This would imply a remarkable ability to determine wind direction when flying in conditions of severely limited visibility¹. We report here an instance of collective insect orientation in nocturnal flight, although in this case, the direction of flight was against the wind.

Our observations were made at Kara in the Niger flood plain in Mali during October, 1973, using radar apparatus

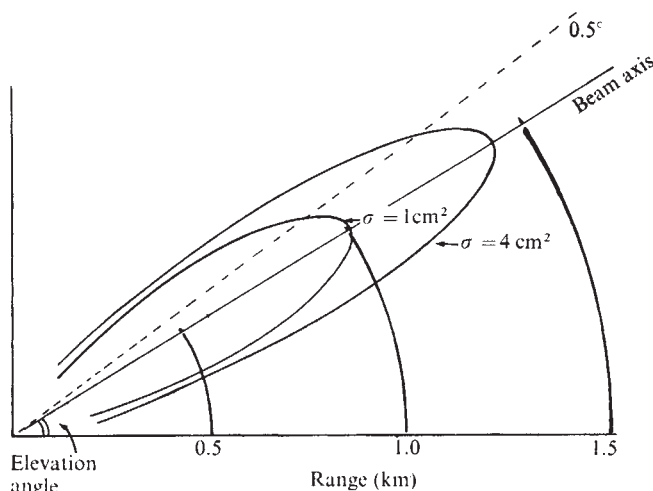


Fig. 1 Typical detection envelopes for two targets of radar cross section (σ) 1 cm² and 4 cm². Targets of these sizes will not be detected outside their respective envelopes. The volume swept out by the envelopes on rotation of the aerial about a vertical axis, and therefore the volumes sampled per revolution for the two sizes of targets, are substantially different. The angular scale in this diagram has been multiplied by 10 to make the effect clear.

modified for entomological observations⁵. The aerial of this radar projects a 'conical' pulsed, microwave beam the axis of which can be set at selected angles of elevation. Rotation of the aerial about a vertical axis causes targets at the appropriate elevations round the radar to be illuminated briefly once per revolution. The resulting radar echoes, if large enough, are registered as 'dots' on a conventional display revolving in synchrony with the aerial. Small targets may be detected at short range and close to the beam axis; larger targets are detectable at greater ranges and further from the axis of the beam. Figure 1 shows typical detection envelopes for two sizes of target. A given airborne volume density of large targets will thus produce more display 'dots' per revolution than the same density of smaller targets.

Measurements made on captive insects indicate that they generally form substantially larger radar targets when the electric vector in the radar wave has a large component parallel to the insect's major body axis^{6,7}. In the case of a radar transmitting horizontally polarised radiation, this means that flying insects will generally present larger 'echoing areas' when flying broadside on to the radar than when end on². A uniform distribution of insects flying with a degree of common orientation in the vicinity of the radar would thus be expected to produce a non-uniform radar display, more 'dots' being seen in the directions from which insects presented predominantly side-on aspects to the radar than in other directions. A pronounced example of this effect is shown in Fig. 2. The distribution of echoes shown in this photograph implies that the insect targets in the vicinity of the radar were predominantly aligned with the 35–215° axis. This 'polarisation' of the echo distribution does not indicate in itself which way along this axis the insects were heading.

Evidence for direction and confirmation of the collective orientation suggested by the polarised display was given by time-lapse photographs of the radar screen. Individual insect echoes were seen to move in the direction of 35° at a ground speed of $\sim 3 \text{ ms}^{-1}$ while the radar echo produced by a freely flying balloon (circled), at the same altitude ($\sim 900 \text{ m}$) and carrying strips of aluminium foil, was displaced towards 215° at $\sim 2 \text{ ms}^{-1}$. The insect heading in this case was clearly against the wind. The aerial density of insects varied from $\sim 50 \text{ per } 10^7 \text{ m}^3$ at 900 m altitude to 20 per 10^7 m^3 at 100 m. The sky was clear with a 7/8 Moon at a bearing of 250°.

The 'polarisation' effect was observed on several nights, being present for 2 or 3% of the observational period (ten nights). The opportunity to provide simultaneous observations

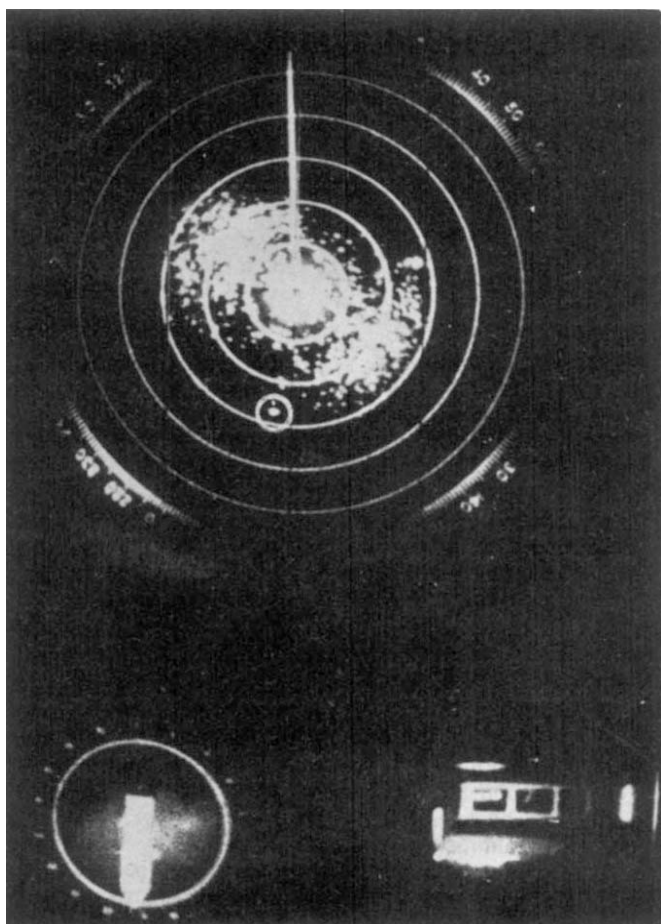


Fig. 2 Photograph of radar screen (1946h, November 3, 1973 at Kara, Mali). Elevation angle 46° , distance between range rings 500 yd. True north is at 0° on the display scale. The echo produced by a freely flying balloon is circled.

of balloon displacement and 'polarisation' did not, unfortunately, recur on these occasions, so that no general implication about heading with respect to wind direction could be inferred. What the observations do show is that some insects regularly demonstrate an ability to adopt a common orientation when flying at night at altitudes of several hundred metres and when separated from each other by distances of the order of 50 m or more.

Information about the identity of insect targets may be gained by measuring the frequency of the modulation produced in their radar echoes by their wing flapping action⁷. Figure 3 shows the distribution of wing-beat frequencies deduced from radar measurements of a sample of the insects responsible for the echoes shown in Fig. 2. Comparison of this histogram with the wing-beat frequencies of other insects found in the

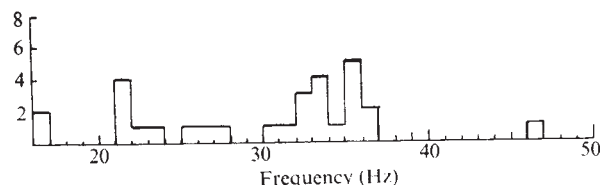


Fig. 3 Histogram showing the distribution of fundamental frequency components detected in radar signatures recorded from targets contributing to the polarised display in Fig. 2. Numbers on the vertical axis indicate the number of signatures containing fundamental frequency components per unit interval of frequency.

area suggests that the echoes may have been caused by grasshoppers, possibly *Trilophidia*, *Oedaleus* and *Locusta*.

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Micromanipulation of stomatal guard cells

THE opening and closing of stomatal pores is controlled by deformations of the guard cells resulting from changes in the pressure relationships between epidermal and guard cells. Under natural conditions such changes are, in turn, controlled by changes in the osmotic concentrations of cell saps or by changes in cell water content, although they could also be controlled by changes in cell wall properties. We have measured the pressures required to bring about or maintain stomatal openings by applying pressures directly within subsidiary cells and guard cells of *Tradescantia virginiana* and *Vicia faba* leaves.

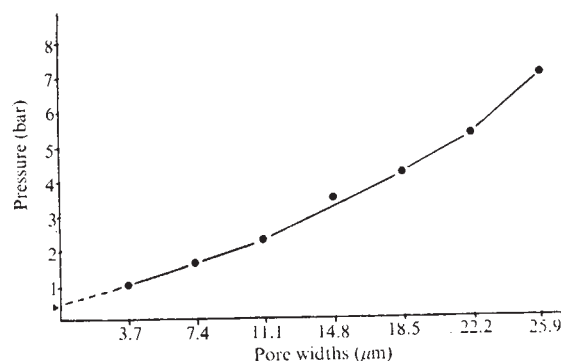


Fig. 1 Pressures applied in subsidiary cells of *Tradescantia virginiana* leaves which caused stomatal closure at various degrees of opening. Each point is the mean of at least 10 measurements with the exception of the values at 3.7 and 25.9 μm where four measurements were made. The pressure values quoted are the total pressures prevailing in the guard cells and not the pressure differentials between epidermal and guard cells. The intercept with the Y axis represents the pressure required to force liquid through the opening of the microneedle. In this species pressures obtained with epidermal strips were of the same magnitude as those with intact leaves. When one of a pair of guard cells was punctured before the application of pressure, the same pressures were required to cause a given degree of opening as with both guard cells intact; this implies that in this species guard cells do not assist each other during movements. Pressures applied in subsidiary cells to close initially open stomata were of the same magnitude as those applied within guard cells to re-open initially almost closed stomata. Until now we have been unable to open completely closed stomata. This observation tends to support Stålfelt's concept of 'Spannungsphase' during which either greater pressures than 10 bar are required before guard cells are deformed; or we must postulate that changes in cell wall properties take place during this phase. The shape of the curve suggests that until an opening of about 10 μm has been reached the deformations of the guard cells do not involve any substantial stretching of the walls in this species. With openings above this size, guard cell walls begin to expand against gradually increasing resistances.

The technique (details will be published elsewhere) consisted of inserting water-filled needles (diameter $<1\ \mu\text{m}$) into the cells and exerting positive or negative pressures using a special syringe attached to the needle. At the same time a transducer was used to measure the magnitude of the pressures, while changes in stomatal pore widths were measured using a microscope with an eyepiece graticule.

Figure 1 shows a summary of the results obtained with *Tradescantia virginiana*. Except during the 'Spannungsphase', operative pressures in the stomatal apparatus were lower than would be expected from some plasmolytically determined osmotic potentials of guard cell saps^{1,2}. Such discrepancies could result from inherent weaknesses in the plasmolytic method. Alternatively, the assumption that guard cells of open stomata (even during steady state openings) are at maximum turgor (in other words, that osmotic potential differences determined plasmolytically cannot be equated to pressure potential differences of guard cells) could be wrong. A mechanism preventing the attainment of full turgor by guard cells can be postulated by reference to the evidence for substantial water vapour loss to the leaf air space from epidermal cells³ and to the outside from guard cells⁴. These possibilities seem to be supported by the comparatively gentle curvature of the line in Fig. 1, suggesting that it is unlikely that these cells were at full turgor. The possibility that guard cell wall properties are unusual and changeable could also be postulated in view of the evidence for ionic migrations between epidermal and guard cells, or between cell walls, the protoplast and vacuoles⁵⁻⁷. These postulates are currently being tested.

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The corneal cones of *Limulus* as optimised light concentrators

In their external shape and optical properties, the crystalline cones of the ommatidia in the compound eye of *Limulus polyphemus* strikingly resemble ideal light collectors. Such devices were developed¹ in a totally different context: for the purpose of optimising the collection of the extremely faint radiation produced in a transparent medium by faster-than-light charged particles (Cherenkov light). Ideal light collectors are ogival-shaped light guides designed to maximise the concentration of diffuse light within a specified angular acceptance. These light guides are optically homogeneous and derive their characteristic properties from the specific shape of the exterior surface which is made specularly reflecting.

Figure 1 illustrates the performance of an ideal light collector. All light rays incident upon the entrance pupil S_1 at an angle $\theta < \theta_{\text{max}}$ are channelled through the exit pupil S_2 after undergoing perhaps one or more reflections. The rays emerge with a large angular spread, approaching 90° to the cone axis. For the

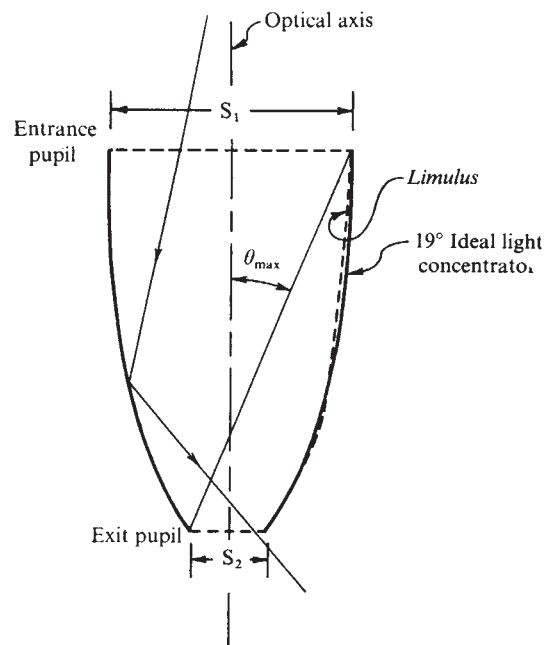


Fig. 1 Profile of ideal light concentrator for maximum angle of acceptance $\theta_{\text{max}} = 19^\circ$. All light rays incident upon the entrance pupil within a cone of aperture θ_{max} are collected and conveyed to the exit pupil. On the right branch of the profile, the shape of a crystalline cone of *Limulus* is also shown.

ideal collector, the concentration factor $x = S_1/S_2$ is related to the angular acceptance θ_{max} by

$$x = 1/\sin^2 \theta_{\text{max}} \quad (1)$$

This performance is characteristic of an optical system with $f/\text{number} = 1/2$, the theoretically lower limit to the relative aperture imposed by geometrical optics. A complementary property of such systems is to exclude any stray light incident at angles greater than θ_{max} .

The qualitative similarity between the profile shape of an ideal light concentrator (Fig. 1) and that of the crystalline cones of *Limulus* became apparent from a micrograph^{2,3} of the anatomy of the compound eye of this Xiphosurid. A quantitative match of the profile shape of several crystalline cones with theoretical shapes corresponding to various acceptance angles θ_{max} showed a very good fit for $\theta_{\text{max}} = 19^\circ$. This is in fact the value of the parameter θ_{max} for the profile shown in Fig. 1, where the comparison with the shape of a typical crystalline cone of *Limulus* is indicated. Cone shapes corresponding to smaller acceptance angles occur near the periphery of the eye.

The simplest optical mechanism by which the *Limulus* cones could function as ideal light concentrators involves total internal reflection of the incident light at the interface between the crystalline cones and the surrounding media. For an optically uniform cone of refractive index $n = 1.54$ (refs 4 and 5) in optical contact with tissue fluid of $n = 1.34$ (ref. 5), the collection of light would be highly efficient, since only a small fraction of light rays would impinge on the internal cone wall at angles in excess of the critical angle (nearly 60°) and leak out. A similar analogy between the ellipsoid portion of retinal cone cells and ideal light collectors has already been noted⁴.

Since the crystalline cones, protruding inward as part of the corneal cuticle, are embedded for about two-thirds of their length in a transparent matrix whose optical properties have never been critically analysed, it was necessary to test the assumptions of our model. The lateral eyes of adult female specimens of *Limulus* were excised and the cornea cleaned of the rhabdomes and pigmented tissue. Throughout all observations, the specimen samples were kept immersed in the original tissue fluid. A portion of the interior visual surface thus prepared is shown in Fig. 2a, where the resemblance of the cone

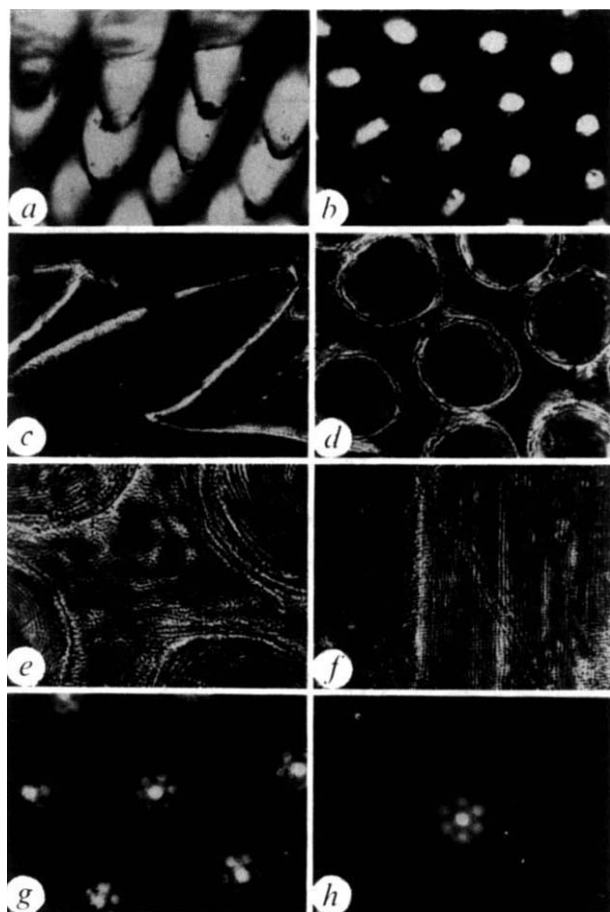


Fig. 2 *a*, Interior surface of the central area of the cornea in the lateral (compound) eye of *Limulus polyphemus*. Fresh specimen, immersed in tissue fluid ($\times 33$). *b*, Interior of the visual surface, as in *a*, when illuminated by a diffuse source from the exterior side of the cornea. The light entering the crystalline cones is channelled to their tips ($\times 33$). *c*, Interference contrast micrograph of the *Limulus* cornea. Fresh section (10 μ m thick) normal to the exterior surface near the periphery of the eye. The section is immersed in tissue fluid. A bright fringe outlines a crystalline cone, whose refractive index is higher than the intracrystalline fill ($\times 55$). *d*, Interference contrast micrograph, as in *c*, of a section parallel to the corneal surface. Bright circular fringes define the cross sections of the crystalline cones ($\times 55$). *e*, Enlarged view of fresh section of the corneal matrix filling the space between crystalline cones. The cross section of the pore canals, filled by tissue fluid is made visible by interference contrast ($\times 133$). *f*, As in *e*, in a longitudinal view. The darker area on the left is part of a crystalline cone ($\times 133$). *g*, Images of a grid (mesh opening 3 mm) placed in front of the visual surface at a distance of 2.6 cm. The images are focused at the tips of the crystalline cones, when the latter are immersed in water. The field of view subtended by each cone belonging to the central region of the eye, is approximately 20° ($\times 48$). *h*, Image obtained at the tip of a lucite macroscopic replica of a *Limulus* cone, having the theoretical profile shape of ideal light concentrators. Lucite cone immersed in water ($\times 38$).

shapes with the profile of Fig. 1 can clearly be seen. The gross light-concentrating properties of the crystalline cones are illustrated in Fig. 2*b*. Even in the absence of pigment, they behave as light guides, concentrating the light incident on the external surface of the cornea on to the tips of the cones, where the rhabdomes are normally attached. Frozen sections ($\sim 10 \mu$ m thick) were then observed with a Baker double focus interference microscope. The crystalline cones preserved their optical identity throughout the entire depth of the cuticle. Their shape is clearly outlined (Fig. 2*c* and *d*) by a bright fringe marking a decrease in refraction index from the interior of the cones to the embedding matrix. All corneal material consisted of cuticle laminations separated by layers of fluid, local variations in the average refractive index, such as that at the cone boundary, arising from variations in the spacing and thickness

of such laminations. The cone interior has a remarkably uniform refractive index, as previously shown⁴. Although the gross refractive properties of the intracrystalline fill can be described in terms of an average measured refractive index $n \sim 1.48$, closer inspection reveals a highly inhomogeneous structure for this tissue (Fig. 2*e* and *f*). It has a loose fabric, permeated by pore canals⁵ running normally to the cuticle laminations. Tissue fluid in the matrix of pore canals seems effectively to constitute a liquid sheath surrounding the highly refracting crystalline cones. The relevant refractive indices for total internal reflection are then those of the cone and tissue fluid respectively, as previously considered⁶.

The phenomenon known as 'pseudopupilla'⁷ is readily explained in our model. All light entering the ommatidium through a cone with angular acceptance θ_{max} is collected and does not re-emerge. In these conditions, the entrance pupil seems a velvety black: the ommatidium behaves as a light sink. Moreover, our simple model of a homogeneous, highly refracting crystalline cone is capable of accounting for the remarkable imaging ability of the crystalline cones observed by many investigators⁷. It is precisely this imaging property (Fig. 2*g*) which prompted earlier attempts to invoke complicated mechanisms for their optical function. A model of the crystalline cone consisting of a lucite light guide ($n=1.5$) with the ideal collector profile immersed in water ($n=1.33$) reproduces the images observed at the tip of an actual cuticular cone of *Limulus*.

Several assumptions have been made in our analysis. The laminated structure of the crystalline cones and the finite thickness of the optical discontinuity at the cone boundaries will make the light collector somewhat inefficient, particularly towards the cone tip. The extent of this light leakage can be appreciated from Fig. 2*b*. In *Limulus* the problem is overcome by encapsulating the distal part of the cone in a sleeve of cells bearing reflecting-pigment, the guanophores⁸.

We conclude that the ommatidia of *Limulus* provide a good example of an optimisation process in nature. Among the possible advantages to an organism that evolves ideal light collectors, the most important is probably the ability to see in dim light conditions.

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Recovery of haemolytic plaque-forming cells after freeze drying

FREEZE drying is generally accepted as the most convenient and often the best method for the preservation of bacteria and viruses. With mammalian cells, the situation is different and

cells such as lymphocytes have to be stored in liquid nitrogen, a method that is cumbersome and inconvenient and which makes transport of such cells particularly difficult. Up to the present, there has to our knowledge been only a single report of successful recovery of function following rehydration of a freeze dried nucleated mammalian cell. This was by Meryman and Kafig in 1959¹ using spermatozoa, but neither they² nor other workers³ have been able to repeat this work. Freeze drying of non-nucleated mammalian cells, such as erythrocytes, has been more successful, however, and both Meryman⁴ and Greaves⁵ have reported the lyophilisation of small numbers of these cells.

The use of cryoprotectants such as dimethylsulphoxide (DMSO) or glycerol are commonplace in the cryopreservation of cells. These substances penetrate the cells and obviate freezing damage probably because of their ability to 'buffer' salt concentration by their colligative properties⁵. When freeze drying is carried out, these substances being non-volatile become concentrated at levels toxic to most cells²⁻⁶. The use of polyvinylpyrrolidone (PVP) in conjunction with foetal calf serum (FCS) has been investigated⁷. PVP does not penetrate the cell and following the previous work it seemed worthwhile to try to use it to protect cells against damage by freeze drying. Here we describe the successful recovery of a lymphocyte function following freeze drying of cells from a medium containing PVP and FCS. The function we chose to investigate is the formation of haemolytic antibody (IgM) as demonstrated by the production of plaques of haemolysis in thin layers of sheep red blood cells (SRBC) by spleen cells from mice previously sensitised with SRBC. This was chosen because of the high sensitivity and specificity of the test.

For each experiment, six female mice (Porton albino) were each injected intravenously with 5×10^8 SRBC. Four days later, the spleens were removed and homogenised by gentle sieving through a nylon sieve. The cells were washed with Medium 199 by centrifugation for 10 min at 190g and were resuspended in Medium 199. The total number of white cells was counted in the normal way in a haemocytometer and the cell density of the suspension adjusted to 5×10^6 ml⁻¹. The number of plaque forming cells (PFC) was estimated by the method of Cunningham and Szenberg⁸. Aliquots of the cell suspensions (5 ml) were distributed in centrifuge tubes, spun for 10 min at 190g and resuspended in one of two previously prepared cryoprotective media. The first was 10% (v/v) DMSO in Hanks solution, the second was 14% (w/v) dialysed PVP (average molecular weight 24,500; Koch-Light Laboratories Ltd., Colnbrook, UK) supplemented with 30% (v/v) FCS in Hanks solution⁷. In preliminary experiments with mouse spleen cells this medium was examined at pH 7.2 and 6.0 and the latter was found to be better for freeze drying. This may have been because the rise in the pH value due to loss of CO₂ from the serum⁹ was balanced by the lower initial pH. In the results presented the pH of the PVP/FCS medium was adjusted to 6.0 as a routine.

For freezing and drying, 10-ml vials (neutral necked vials, Specification no. 1 (M) 3479, Johnson and Jorgensen Ltd, London) were used, freezing being carried out in 1-ml amounts and freeze drying in 0.1-ml amounts. The samples were cooled slowly (approximately 2° C min⁻¹) on the pre-cooled shelves (-40° C) of the EF6 freeze drier (Edwards High Vacuum Ltd)

Table 1 Recovery of total number of mouse spleen cells and plaque-forming cells after freezing and freeze drying in 10% DMSO

Cell suspensions	Total white cell recovery		PFCs	
	No. cells (ml ⁻¹ × 10 ⁶)	%	Total no.*	%
Control	5.0	100	1,380	100
Frozen and thawed	3.9	78	147	10.6
Freeze dried and rehydrated	0.75	15	0	0

* Each value is average of counts in two chambers.

Table 2 Recovery of total number of mouse spleen cells and PFCs after preparative manipulation and after freezing and freeze drying in the medium containing 14% PVP and 30% FCS

Cell suspensions	Total white cell recovery		PFCs	
	No. cells (ml ⁻¹ × 10 ⁶)	%	Total no.*	%
Control	5	100	1,090	100
Untreated cells after preparative manipulation	2.3	46	510	46
	1.9	38	465	42
	1.7	34	495	45
Frozen and thawed	1.9	38	155	14
	2.2	44	145	13
	1.5	30	130	11
Freeze dried and rehydrated	2.1	42	130	11
	1.5	30	150	13
	2.3	46	170	15

Control suspension contained cells in Medium 199, the cell density being adjusted to 5×10^6 cells ml⁻¹, 5 ml being examined. The cell suspensions in the PVP/FCS medium were divided into three groups and treated as follows. First, cells from three 5 ml samples were centrifuged again and resuspended in Medium 199. This was devised as a control for the manipulation other than freezing and drying. Second, cell suspensions from three 5 ml samples were distributed in vials and frozen in 1 ml mounts; this was the control for freezing without drying. Third, suspensions from three 5 ml samples were dried in 0.1 ml amounts. After rehydration, pooling in 5 ml amounts and subsequent centrifugation, the pellets of these samples were resuspended in Medium 199. All the experimental groups were examined in triplicate.

* Each value is average of counts in two chambers.

down to -38° C and -40° C and then removed and thawed quickly in a water bath at 37° C (1-ml samples) or freeze dried (0.1-ml samples). For the latter, as soon as a vacuum of 0.07-0.09 torr had been obtained, the temperature of the shelves was raised slowly (2 h) to -20° C, and drying carried out at this temperature for 2 h. The vials were then cooled again to -40° C, dried for an additional 20 h and sealed under vacuum with rubber stoppers. This technique resulted in a residual moisture of 8-10% which was determined by a microdrying method¹⁰. To rehydrate the cells, 0.1 ml of cold Medium 199 was added on the surface of the freeze dried cake, the vials being kept at -40° C. After the medium had frozen the vials were removed from the cold shelves and stood at room temperature to allow the ice to melt. Thus the cake was rehydrated at the temperature of melting ice. The thawed and rehydrated samples were pooled in 5.0-ml amounts, centrifuged and resuspended in Medium 199. The total white cell counts and the number of PFCs were then determined.

Table 1 shows the results of tests when DMSO was used as a cryoprotectant. 78% of the total number of cells was recovered from the freezing and thawing process, while only 10% of the original PFC survived. When the cells were rehydrated following freeze drying, only 15% of the total cells were found and not a single PFC. These results are typical of our experience with the use of DMSO. It is a satisfactory cryoprotectant as far as freezing is concerned but it is toxic when used in a process involving freeze drying.

Table 2 shows the results obtained in an experiment in which PVP and FCS were used to protect the cells. In several experiments, we had found a substantial loss in the total cells and PFCs during the manipulations before either freezing or freeze drying. Such a loss is shown in Table 2. In spite of this, however, it is clear that 30-40% of the total cells have been recovered from the complete process, while 11-15% of the PFC have come through. If the loss of cells due to the manipulations is taken into account, the recovery of total cells from the freeze drying process becomes nearly 100% and the recovery of PFC nearly 30%. No change of total cell numbers or PFC was noticed after 7 d storage at 4° C.

Having thus established that cells dried in the presence of DMSO would not produce haemolytic plaques whereas those

freeze dried from PVP would, we decided to investigate various properties of the plaques. In particular, we wished to know whether the plaques were produced by antibody; whether antibody was secreted or diffusing; or whether antibody synthesis was involved.

The results shown in Table 3 pertain to the first two questions. The total abolition of plaques by azide, found on more than one occasion, shows that plaque formation is energy dependent and thus not the result of simple leakage of preformed material from damaged cells. That the plaques are formed by IgM antibody is shown by the facts that no plaques are formed in the absence of complement, that plaque formation is inhibited by dithiothreitol and that anti- μ -chain serum which is immunologically specific for the μ chain of mouse IgM molecules also blocks plaque formation. Thus, the results show that plaques are formed by IgM molecules secreted from cells by a process requiring energy and, as haemolysis occurs when sheep red blood cells are the target cell and not when chicken erythrocytes are used as a target, it is clear that the required specificity of the antibody exists and demonstrates that the plaques are due to specific IgM and are not an artefact.

Table 3 Haemolytic plaque formation by rehydrated freeze dried cells

Experiment	Total white cell count (ml ⁻¹ × 10 ⁶)		PFCs*					
	1	2	1	2	2a	2b	2c	2d
Control	5.0	4.8	435	1,100				
		4.7		965				
		5.1		1,035				
Untreated cells after preparative manipulation	2.3	1.8	210	425				
		2.8		485				
		3.0		375				
Freeze dried and rehydrated tested as Exp. 1	1.4	—	0	—				
		1.1		—				
		1.4		—				
Freeze dried and rehydrated	1.8	1.1	345	135	0	0	0	0
		2.0		130				
		1.7		100				

Experimental details are as given in Table 2. In experiment 1, 10⁻¹ M azide was added to one group of chambers. In experiment 2, the following variations were used: 2a, the chambers contained no complement; 2b, the chambers contained chicken red blood cells in place of sheep red blood cells normally employed; 2c, the chambers contained 1 drop of 1:2 dilution of rabbit anti-mouse μ -chain serum; 2d, the chambers contained 10⁻¹ M dithiothreitol.

* Each value is average of counts made in two chambers.

Further information on the level of cellular integrity was sought by the use of an inhibitor of protein synthesis, in this case puromycin. This antibiotic causes chain termination by forming a peptide bond with the nascent polypeptide forming on the ribosome¹¹. In a preliminary experiment, puromycin at 10⁻⁴ M inhibited PFCs by about 60%. A further experiment was set up, the results of which are shown in Table 4, from this it can be seen that puromycin at 10⁻² M failed to give complete inhibition of PFC in either the control or the prefrozen and manipulated samples, giving in fact a reduction to around 17% and 10% respectively (with 10⁻³ M puromycin, the figures were 70% and 60%). In the case of freeze dried and reconstituted cells, there was virtually no inhibition at 10⁻³ M but the 10⁻² M concentration gave a reduction to about 40%. These results would seem to indicate that there has been a selection of non-puromycin sensitive cells following freeze drying but that there is still a proportion of these cells in which some degree of protein synthesis, that is, production of nascent polypeptide chains, is occurring.

We have also considered the freeze drying aspects of this problem. The moisture levels reported here are high relative to the standards accepted in microbiology. It should be noted, however, that the 'microdrying' method for moisture determination used in the present study is based on the loss in weight of a

Table 4 Effect of puromycin on plaque formation by mouse spleen cells

Experiment	Total white cell count (ml ⁻¹ × 10 ⁶)	PFCs*		
		Normal	10 ⁻³ M puromycin	10 ⁻² M puromycin
Control	5.6	5,740	3,665	1,150
	6.3	6,135	4,335	1,020
	6.7	5,485	4,165	870
Untreated cells after preparative manipulation	4.8	2,835	1,725	285
	5.0	3,540	2,485	475
	4.4	3,335	2,590	205
Freeze dried and rehydrated	3.4	1,155	1,180	535
	4.7	1,685	1,295	565
	3.9	1,245	1,190	530

* Each value is average of two counts made in two chambers.

5–10 mg sample on drying in a vacuum at an elevated temperature (80–100° C) and is said to give considerably higher moisture contents than the so-called American standard method¹⁰. Further, we have demonstrated experimentally that even at moisture levels of 2.7% as determined by the Baker method¹⁰, PFC can still be found although at lower recovery rates, 25% of PFCs in control compared with 45% at 11% moisture. The inability of hitherto reported mammalian cells to withstand dehydration to a water content below about 25% (refs 12 and 13) renders our results even more encouraging.

We have also been able to show that freeze dried cells with a residual moisture content of around 5% can be stored for up to 3 weeks at room temperature and still retain their capacity to produce plaques.

The number or proportion of PFCs recovered following freeze drying is currently very variable and at present we have no satisfactory explanation of this fact. Analysis of results from six separate experiments and two storage experiments shows that the results were variable. Geometric means and standard deviations were calculated from log transformed data. The geometric means found (shown with their s. d.) varied from as few as 6.1^{+4.9}_{-2.7} PFCs recovered from 197.9^{+27.6}_{-24.2} PFCs to as many as 35.2^{+4.9}_{-4.4} PFCs from 37.2^{+7.9}_{-6.5} PFCs. Most results, however, are not as extreme as these and suggest that a mean recovery of 10–15% of the PFCs in the original spleen cell suspension is found after all manipulative and freeze drying losses. The loss due solely to freeze drying and rehydration is difficult to assess now. The puromycin experiments indicate that some selection of cell population occurs during these processes.

Our experiments suggest the inadequacy of the more commonly used intracellular cryoprotectant agents, such as glycerol and DMSO, for protecting cells against the effects of freeze drying. These agents concentrate within the cell during the sublimation of the water and reach levels that are toxic to the cells^{2,6}. With DMSO in our study, not a single PFC survived. It would seem, therefore, that only those mammalian cells which can survive freezing without glycerol or DMSO may be potential candidates for freeze drying. The extracellular protective medium used here has recently been developed by us⁷ and it has been shown to protect effectively human lymphocytes against freezing damage.

The haemolytic effects seen in our experiments are shown to be due to the active secretion of complement-fixing, antigen-specific IgM molecules. At least some cells seem to be involved in polypeptide synthesis on the ribosomes. Thus, a degree of functional integrity of the cell has been demonstrated and we believe therefore that the work we have described represents a novel and important step towards the recovery of fully functioning nucleated mammalian cells following freeze drying. It may be noted also that for the first time drying-rehydration injury has not been of a higher degree than freezing-thawing injury. So far even in the preservation of micro organisms, freeze drying has always been more deleterious than freezing¹⁴.

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Evidence against interconversion of microtubules and filaments

MICROTUBULES, hollow cylindrical structures of diameter ~ 250 Å and variable length, consisting of 13 protofilaments (diameter ± 40 Å), are now generally accepted as an essential constituent of most eukaryotic cells. They have been implicated in such diverse functions as: maintenance of cell shape, intracellular transport and organisation, movement (particularly slow morphogenetic movement) and control of cell surface topography (for review see ref. 1).

In general, cytoplasmic microtubules are intermingled with filaments (diameter ~ 100 Å), and it has been suggested that they are two components of one functional system. The (supposedly) rigid microtubules would then serve as rails along which organelles are transported through interconnecting filaments². Numerous observations both *in vivo* and in cell culture systems have shown that disruption of microtubules is followed by the appearance of large amounts of filaments often arranged in tightly packed bundles^{3–7}. This has usually been interpreted as an interconversion of microtubules into filaments (refs 3 and 8). Other authors, however, favour the idea that depolymerisation of microtubules would result in a redistribution of existing filaments from a dispersed status to an aggregated form⁹.

Unfortunately, the very nature of these structures does not make it feasible to perform accurate morphometric studies which could solve the problem. We have compared the effect of different antitubulins at different dose levels and with prolonged incubation times, in particular the vinca alkaloids, considered to be very useful in this context, since these drugs are able to precipitate microtubular protein as crystalline aggregate at higher dose levels. We have tested the following hypotheses: (1) If microtubules and filaments are composed of the same precursor protein then there should be an inverse relationship between filament whorls and 'microtubular' crystals in cells treated with vinca alkaloids. (2) If there is a redistribution (aggregation) of filaments after microtubular disruption then these filaments should be absent in the zones between the bundles. (3) If the occurrence of bundles is due

to enhanced synthesis of filaments then this should be largely inhibited by inhibition of protein synthesis.

Cells from a C3H mouse embryonal cell line (MO; ref. 10) show contact inhibition of both movement and division, and form transparent sheets of large polygonal cells at confluency. When these cells were treated with antitubulins (colchicine, vinblastine and vincristine) they lost their characteristic stretched appearance and assumed a flattened round form. This coincided in time with the disappearance of microtubules at the ultrastructural level, and with a total loss of intracellular orientation and compartmentalisation. This was most obvious in the organisation of the Golgi field which has a perinuclear position around the centrioles in untreated cells and 'explodes' completely after microtubule dissolution, with individual Golgi organelles being distributed over the entire cytoplasm; and the centrioles often assume a peripheral position. Both subplasmalemmal microfilaments and 100 Å filaments were obviously unaffected. The foregoing took place within 10 to 80 min, depending on the dosage used ($100\text{--}0.04$ $\mu\text{g ml}^{-1}$). After 2 h a proliferation of smooth endoplasmic vesicles occurred, which later subsided, followed by a general hypertrophy of the rough endoplasmic reticulum and the appearance of annulated lamellae. Increased amounts of 100 Å filaments were observed after only 5 h, bundles and whorls of which continuously increased in size and number, and reached a maximum after 24–48 h (Fig. 1a). The zones between the bundles still contained individual filaments.

When cells were treated for prolonged periods (24–48 h) and subsequently replaced in normal growth medium they regained their normal stretched appearance within 24 h. At the ultrastructural level this coincided with the reappearance of microtubules, beginning after about 4 h, and consequent intracellular reorganisation. Within 24 h the centrioles re-assumed their normal perinuclear location in the centre of the regrouped Golgi zone. Filament whorls diminished in size and number, however, at a very slow rate, large aggregates still being observed after 48 h. Both the rate of appearance of filament accumulation after treatment and the disappearance after discontinuation of treatment can hardly be explained by either interconversion of microtubules in filaments or a mere redistribution of pre-existing filaments.

We tried to check the most likely explanation: an enhanced synthesis of filaments after microtubule dissolution. Cells were pretreated with cycloheximide (10 $\mu\text{g ml}^{-1}$) followed by colchicine (1 $\mu\text{g ml}^{-1}$) after 16 h. In this situation (which produced a 95% inhibition of labelled leucine incorporation into acid precipitable material; M.D.B., unpublished), the cells reacted in the same way, except for the total lack of filament whorls even after 48 h of treatment (Fig. 1b). Individual filaments could, however, be observed throughout the cytoplasm. Additional evidence against interconversion of microtubules and filaments was obtained by treating the cells with vinca alkaloids. Both vinblastine and vincristine produced effects identical to those of colchicine, filament bundles occurring in the same amount at all doses tested ($10\text{--}0.1$ $\mu\text{g ml}^{-1}$). At doses above 1 $\mu\text{g ml}^{-1}$, however, an additional feature appeared: the occurrence of large crystalline aggregates, with a tubular substructure, most prominent at 10 $\mu\text{g ml}^{-1}$. These structures, known to be composed of tubulin^{8,11}, did not interfere in any way with the concomitant presence of filament whorls (Fig. 1c).

These results show that neither interconversion of microtubules in filaments nor redistribution of pre-existing filaments are sufficient explanations for the appearance of large filament aggregates in MO cells after microtubule dissolution. Moreover, it can hardly be attributed to a side-effect of the drug used, since it is produced by all known antitubulins regardless of their often totally different chemical nature. The most likely explanation is obviously an excessive synthesis of filamentous protein.

Although the function of these filaments has not yet been completely elucidated, they always occur in close connection

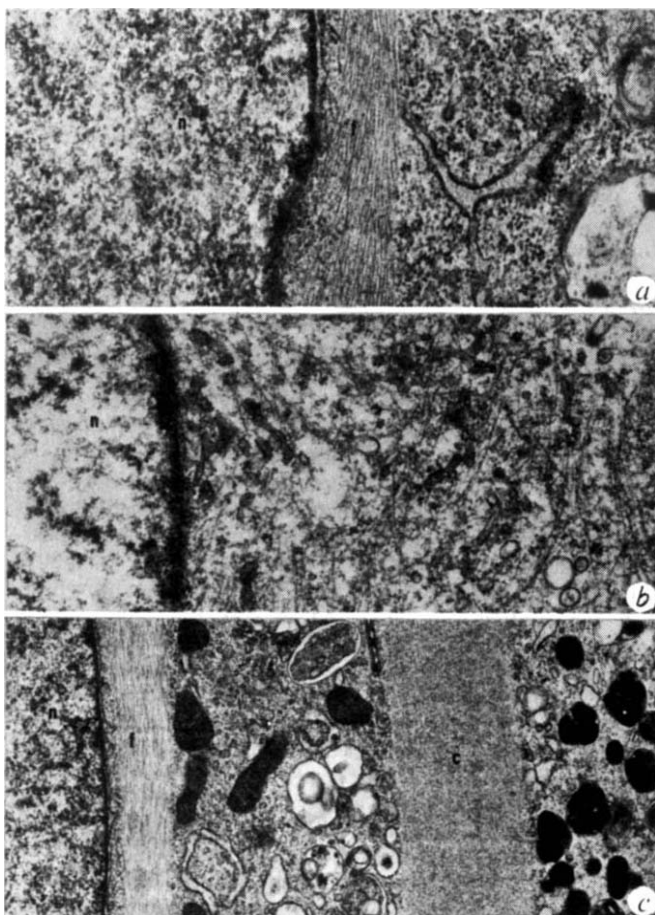


Fig. 1 *a*, Bundle of filaments (f) in the vicinity of the nucleus (n) in a MO cell treated for 24 h with colchicine ($1 \mu\text{g ml}^{-1}$) ($\times 26,000$). *b*, Perinuclear region in a MO cell treated for 24 h with cycloheximide ($10 \mu\text{g ml}^{-1}$) followed by colchicine ($1 \mu\text{g ml}^{-1}$) after 16 h. Filament bundles are completely absent although individual filaments are present. ($\times 22,000$). *c*, Perinuclear region in a MO cell treated for 24 h with vinblastine ($10 \mu\text{g ml}^{-1}$). Both filament bundles (f) and crystalline structures (c) are present. ($\times 11,000$). The cells were cultured in Eagle's minimum essential medium supplemented with 10% foetal bovine serum. For observation with the phase contrast microscope and subsequent fixation for electron microscopy the cells were cultured in Falcon plastic Petri dishes (6 cm ϕ). At the appropriate moment the medium was decanted and the cultures washed twice with saline at room temperature. The cells were fixed with 5% glutaraldehyde in 0.1 M sodium cacodylate at room temperature for 15 min and postfixed with a mixture of 4% glutaraldehyde and 1% osmium tetroxide at 0°C . After dehydration in a graded alcohol series the cells were embedded in epon. Under the phase contrast microscope individual cells were selected for further processing for electron microscopy. A minimum of 50 cells was examined per treatment group, each cell being sectioned in a semi-serial fashion from bottom to top, to exclude major sampling errors.

with microtubules in untreated cells. On the other hand, they frequently seem to make contact with organelles such as mitochondria and lysosomes^{12,13}. This has led to the supposition that filaments, together with microtubules, could be responsible for the intracellular directional movement of these organelles^{2,14,15}. A possible hypothesis is that the increased production of filaments after microtubule dissolution could be due to some feedback mechanism whereby the loss of one component of the system (microtubules) is, to some extent, overcome by the hypertrophy of the other component, which could presumably take over some of the lost functions.

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Morphine antagonises action of prostaglandin in neuroblastoma and neuroblastoma $\times$ glioma hybrid cells

WORK on cell lines derived from tumours of the nervous system has been carried out to establish models for neurones and glial cells. Clonal cell lines derived from mouse neuroblastoma C1300 possess many properties characteristic of neurones¹⁻¹¹. While the neuroblastoma cells increase in their intracellular levels of adenosine 3',5'-cyclic monophosphate (cyclic AMP) in the presence of prostaglandin E_1 (PGE₁) (refs 12 and 13), acetylcholine¹⁴ and adenosine¹⁵, rat glioma cells¹⁶ do so when exposed to noradrenaline^{17,18} or PGE₁ (ref. 19).

Hybrids between mouse neuroblastoma and rat glioma cells display remarkable neuronal properties. In contrast to their parental lines, they contain choline acetyltransferase^{20,21}. They are rather large cells capable of extending long processes; they fire action potentials in response to depolarisation by electrical current or acetylcholine²¹, they contain clear and dense core vesicles²² and have dopamine- β -hydroxylase activity⁶; they increase their content of cyclic AMP in the presence of PGE₁ (ref. 19).

In the course of our studies on the action of PGE₁ on neuroblastoma cells and their hybrids, we investigated²³ the potency of substances, which had been reported^{24,25} to interfere with the influence of prostaglandins. Among these we examined morphine²⁶, as the opiate is known to inhibit the contractions of intestinal muscle^{25,27,28} evoked by PGE. Here we report that morphine antagonises the stimulatory action of PGE₁ on the level of cyclic AMP in neuroblastoma and neuroblastoma $\times$ glioma hybrid cells. While this work was in progress²⁸, Collier and Roy reported that morphine inhibited the stimulation by PGE₁ of the formation of cyclic AMP in rat brain homogenates²⁹.

The neuroblastoma¹³ and neuroblastoma $\times$ glioma hybrid¹⁹ lines which are most responsive to PGE₁ were cultured in plastic dishes 10 cm in diameter. For experimental incubation, the cells were kept for 10 min at 37°C , at varying concentrations of morphine in the absence or presence of $10 \mu\text{mol l}^{-1}$ PGE₁ as described previously²³. Morphine hydrochloride, dissolved in Dulbecco's modified Eagle's medium, was added (50 μl) to 5 ml

of incubation medium. After the incubation, medium and cells were separately assayed for cyclic AMP^{23,30}. The data are referred to cellular protein³⁰.

Curve *a* of Fig. 1 demonstrates that morphine at concentrations between 0.1 and 100 $\mu\text{mol l}^{-1}$ inhibits the increase of cyclic AMP levels evoked by PGE_1 . Up to 12% of the total cyclic AMP thus formed is found in the incubation media (Fig. 1, curve *b*). Release of cyclic AMP into the surrounding media has been described previously^{23,31}. As the fraction of cyclic AMP released from the cells is always low in these conditions, however, the corresponding data are omitted from the subsequent figures. The inhibitory action of morphine decreases at concentrations above 0.1 mmol l^{-1} (Fig. 1, curve *a*). We observed²³ a qualitatively similar phenomenon using another antagonist of PGE_1 , 7-oxa-13-prostinoic acid²⁴. It remains to be established whether or not the two phases of Fig. 1, curve *a* correspond to the sites of stereospecific and non-stereospecific opiate binding³². In cells not stimulated by PGE_1 , no effect of morphine on the level of cyclic AMP is noticed (Fig. 1, curve *c*). Again, only about 10% of the total cyclic AMP is found in the medium.

On exposure to PGE_1 the level of cyclic AMP reached in neuroblastoma $\times$ glioma hybrids is considerably higher than in neuroblastoma line N4TG3. The cells are more sensitive to morphine than N4TG3 (Fig. 2, curve *a*). The 50% inhibitory concentration (IC_{50}) is 5 $\mu\text{mol l}^{-1}$ for the hybrids compared with 400 $\mu\text{mol l}^{-1}$ for the neuroblastoma line. In three further series of experiments with N4TG3, however, the IC_{50} was in the range of 5–20 $\mu\text{mol l}^{-1}$. The causes for this scattering of morphine-sensitivity values are under investigation. IC_{50} values of 5 $\mu\text{mol l}^{-1}$ were also reported for the adenylyl cyclase system in brain homogenates²⁹. In considering the cell culture system as models for nervous tissues in intact animals, it is important that it is also micromolar concentrations of morphine in brain which exert an antinociceptive effect (data recalculated from ref. 33). Similar to the neuroblastoma line, the hybrids show no sensitivity to morphine in the absence of PGE_1 (Fig. 2, curve *b*).

The morphine analogue naloxone is known to antagonise the action of morphine without causing morphine-like effects itself³⁴. As seen in Fig. 3, naloxone exerts such an effect also on

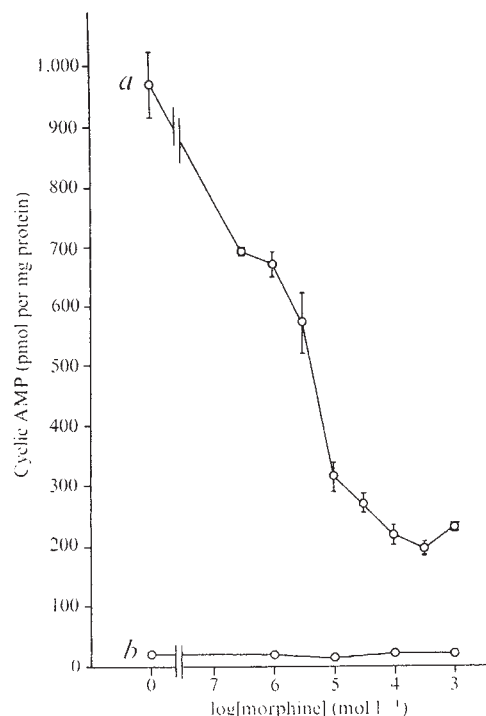


Fig. 2 Inhibition by morphine of the stimulation by PGE_1 of cyclic AMP formation in clonal neuroblastoma N18TG2 $\times$ glioma C6-BU-1 hybrid line 108CC15. A brief description of the cells has been given elsewhere^{19,20}, a detailed report is in preparation. Passage number 14; 5.9×10^6 viable cells per plate; viability 85%. cyclic AMP in cells; *a*, 3 $\mu\text{mol l}^{-1}$ PGE_1 ; *b*, no PGE_1 .

neuroblastoma cells. The inhibitory effect of 0.1 mmol l^{-1} morphine is abolished by 1–10 $\mu\text{mol l}^{-1}$ naloxone (Fig. 3, curves *a* and *b*). Furthermore, in concentrations above 0.1 mmol l^{-1} , naloxone itself causes an elevation of the level of cyclic AMP (Fig. 3, curve *c*), and, in combination with PGE_1 , potentiates the action of the latter (Fig. 3, curve *a*). This effect is even greater when morphine is also present (Fig. 3, curve *b*). This result suggests that in the concentration range of 0.1–1 mmol l^{-1} morphine and naloxone are not antagonists but rather act synergistically (Figs 1 and 3). Work is under way to elucidate this phenomenon. In Fig. 3, the biphasic shape of curves *a* and *b*, together with the notion that naloxone and PGE_1 increase the levels of cyclic AMP well above that seen when PGE_1 is present alone, indicates two different binding sites for naloxone. The similarity of the effects exerted by morphine and naloxone at high concentrations and their synergism might suggest that the drugs act at the same low affinity binding sites³² and that the specificity of binding is relatively low.

This study adds another item to the increasing list of differentiated nervous functions found in cell lines derived from the nervous system and suggests the suitability of such cells as model system for the investigation of opiate action. In particular, the antagonism between PGE_1 and morphine in the cultured cells parallels strikingly the antagonism observed in intestinal contraction^{25,27,28}. Although in both N4TG3 and 108CC15 the increase of the intracellular levels of cyclic AMP is inhibited by morphine, the hybrid line is more promising as a model system. It is more sensitive to PGE_1 and to morphine than N4TG3. The inhibitory effect of morphine is non-competitive as it is not reversed at high PGE_1 concentrations (30 $\mu\text{mol l}^{-1}$). This is more than 300 times the dose at which PGE_1 elevates cyclic AMP to half maximal value³⁵. Furthermore, not all cell types sensitive to PGE_1 are also sensitive to morphine, as shown in a glioma and glioma $\times$ fibroblast hybrid line³⁵. Like PGE_1 , noradrenaline increases the level of cyclic AMP in these cells. Furthermore, this response is not inhibited by morphine. Thus, the antagonistic action of morphine may be restricted both with respect to the cell type and to the hormone affected.

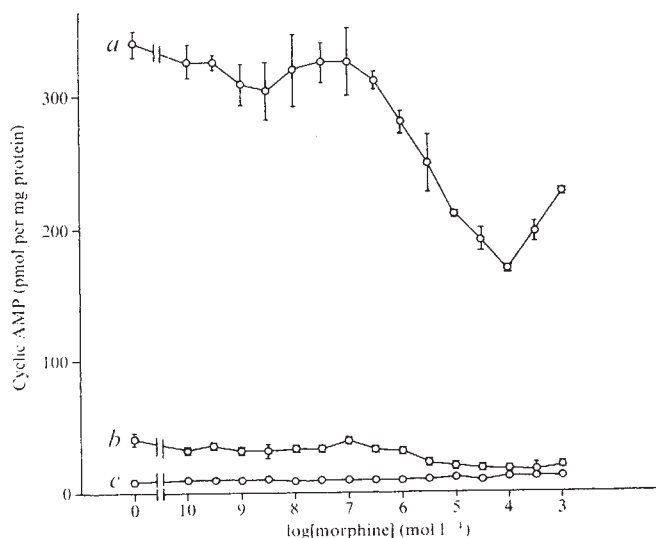


Fig. 1 Inhibition by morphine of the stimulation by PGE_1 of cyclic AMP formation in neuroblastoma clone N4TG3. N4TG3 is a 6-thioguanine resistant mutant²⁰ of a clonal line derived from neuroblastoma C1300 (ref. 3). At varying concentrations of morphine, the cells are incubated in the presence and absence of PGE_1 . Subsequently, the cyclic AMP formed is measured. s.d. are given together with the mean value of three parallel incubations. Data without standard deviations are obtained from single plates. 17.6×10^6 cells per plate, passage number 27, viability (exclusion of nigrosin) 96%. *a*, cyclic AMP in cells + medium, 10 $\mu\text{mol l}^{-1}$ PGE_1 ; *b*, cyclic AMP released into the incubation medium, 10 $\mu\text{mol l}^{-1}$ PGE_1 ; *c*, cyclic AMP in cells plus medium, no PGE_1 .

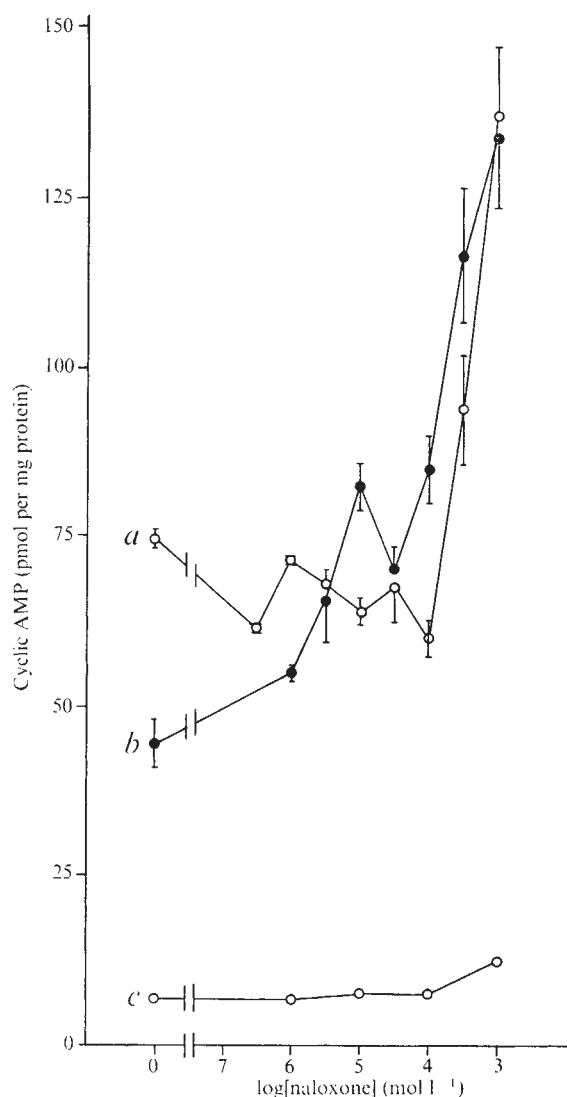


Fig. 3 Naloxone prevents the inhibition by morphine of the cyclic AMP formation stimulated by PGE₁. 16.1×10^6 viable N4TG3 cells, passage number 29, viability 91%. *a*, $3 \mu\text{mol l}^{-1}$ PGE₁; *b*, $3 \mu\text{mol l}^{-1}$ PGE₁ + 0.1 mmol l^{-1} morphine; *c*, without PGE₁ and morphine. The inhibitory effect of morphine in the absence of naloxone is evident, when the points at 0 mol l^{-1} naloxone of curves *a* and *b* are compared.

The study of the clonal cell lines sensitive to morphine may have provided a first glimpse at the mechanisms involved in opiate action. The advantage of the system is its simplicity. The cell masses of a clone are relatively homogeneous compared with the complex mixture of cells in brain. Roy and Collier²⁹ studying rat brain homogenates found an adenylyl cyclase which is stimulated by PGE₁; the stimulation is inhibited by morphine. The use of the cell-free system may be limited by the high background activity of adenylyl cyclase in the absence of PGE₁ and by the very low sensitivity to PGE₁. The rat brain homogenates are at least 300 times less sensitive to PGE₁ than N4TG3 cells^{29,35}. Nevertheless the major findings—the antagonism between PGE₁ and morphine and the involvement of the adenylyl cyclase system in morphine action—are the same with both approaches and thus the conclusions drawn are similar.

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Action potentials induced in slow muscle fibres by partial denervation

FROG slow muscle fibres are usually unable to generate action potentials^{1,2}. It has been demonstrated, however, that this ability is acquired after denervation^{3,4}, and it has been suggested that the inhibitory effect of the small motor axons on the slow fibre membrane results from the release of a 'trophic' substance^{4–6}. Since slow muscle fibres are usually innervated by more than one motor axon¹, it was important to find out, whether the action potential is induced after partial removal of this 'trophic' influence. Our results show that this is indeed the case.

The experiments were performed on the right pyriformis muscle of *Rana temporaria*. This muscle is usually innervated by spinal branch IX; it has, however, been observed⁷ that occasionally the muscle receives additional motor axons from spinal branch VIII. One of these branches, usually IX, was cut in the pelvis. After the operation the frogs were kept at room temperature (about 19°C), and 11–13 d later the pyriformis muscle was removed, together with its nerve, for

electrophysiological examination⁸. The slow fibres of the piriformis muscle are 10–15 mm long and their length constant is of the same order of magnitude⁹. With a single insertion of the microelectrode measuring potential (about half-way between the tendons), the whole fibre can therefore be explored. Rectangular current pulses were applied to a second microelectrode inserted at a distance of 50–100 μm and the slow fibres were identified by their electrical properties⁹. The nerve was mounted on two pairs of platinum electrodes for stimulation of motor axons and determination of their conduction velocities⁸. Ringer's solution had the following composition (mM): NaCl 110.4; KCl 2.5; CaCl_2 7.2; Tris 5.0. All measurements were performed at 8–10°C.

In all, sixteen muscles were examined after transection of spinal branch IX (14 muscles) or VIII (2 muscles); twelve muscles proved to be completely denervated, while in the remaining four muscles variable proportions of denervated muscle fibres were identified. The slow fibres could be subdivided into three groups. Three fibres (group 1) were completely denervated, presumably because they were originally innervated only by the spinal branch which had been cut, showed action potentials as expected (Fig. 1A). Nine fibres (group 2) showed action potentials, generally of smaller amplitude but in contrast to group 1 they responded with an end-plate potential to nerve stimulation (Fig. 1B). Three fibres (group 3) were also innervated but did not produce action potentials, and therefore seemed to be normal slow fibres.

Similar results were obtained in a second series of experiments, in which one small branch of the piriformis motor nerve was cut 1–2 mm from its entry into the muscle. Among the 43 slow fibres identified 8–15 d later 26 belonged to group 2, being innervated and yet capable of generating action potentials. Eight fibres were completely denervated and produced action potentials (group 1), while nine fibres seemed to be normal (group 3).

It is important to eliminate the possibility that those slow fibres (group 2) showing both end-plate and action potentials were in the early stage of reinnervation after having been completely denervated. Reinnervation by regenerated motor axons could be excluded, because inspection of the operation site revealed that the proximal and distal nerve stumps were always clearly separated; furthermore there would not have been enough time for regeneration, at least in those experi-

ments in which the spinal branch had been cut. The alternative possibility, that reinnervation of totally denervated fibres had taken place by collateral sprouting from intact axons also seems unlikely in view of the short period (sometimes only 8 d) that had elapsed after the operation. Furthermore the characteristics of the end-plate potentials from the group 2 fibres also suggested that they occurred at residual 'old' end-plates rather than newly formed ones. Thus their amplitudes were usually smaller (mean 18 mV) than normal (30 mV) but not markedly different, and they occurred with normal latencies. Moreover, the majority of fibres (33 out of 35) were innervated by a single slow axon (conduction velocity smaller than 5.2 m s^{-1}).

Our experiments demonstrate that two or more small motor axons are necessary to suppress the action potential mechanism in slow muscle fibres. It is conceivable that each individual axon exerts its effect locally as has been shown for the neural control of acetylcholine sensitivity in frog twitch fibres¹⁴. In this case the transformation of the slow fibre membrane could be restricted to the vicinity of degenerating end-plates, while the membrane remains unchanged near normal end-plates. It may also be that cutting one or more small motor axons leads to a general reduction of the inhibitory effect such that sodium-channels are simultaneously built into the membrane along the entire length of the fibre. At present we cannot distinguish between these two possibilities and more work is needed to understand how the small motor axons exert their 'trophic' effect on slow muscle fibres. It seems clear, however, that a reduction in size of the end-plate potential is not directly responsible because Botulinum toxin markedly reduces the end-plate potential without inducing an action potential¹⁵.

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British anti-Lewisite and organomercury poisoning

2,3-DIMERCAPTOPROPANOL ($\text{CH}_2(\text{OH})\text{CH}(\text{SH})\text{CH}_2\text{SH}$; BALH₃) is generally used in the treatment of mercury poisoning to help remove mercury from the body^{1–3}. It has been reported, however, that use of BALH₃, together with, or shortly after injection into laboratory animals of inorganic mercury^{4–6}, methylmercury^{7–8}, or 'phenylmercury'^{7–9} compounds results in a different initial distribution of mercury in the body than when the compounds are injected alone. For inorganic mercury, the results can be explained in terms of the timing and dosage of BALH₃ (ref. 6). For both methyl and phenylmercury compounds with BALH₃, the mercury content of the brain is greatly enhanced^{7–9}. Although the initial rapid (24 h) distribution of mercury in the animals treated for methylmercury poisoning with BALH₃ is similar to that achieved after 8 d in the absence

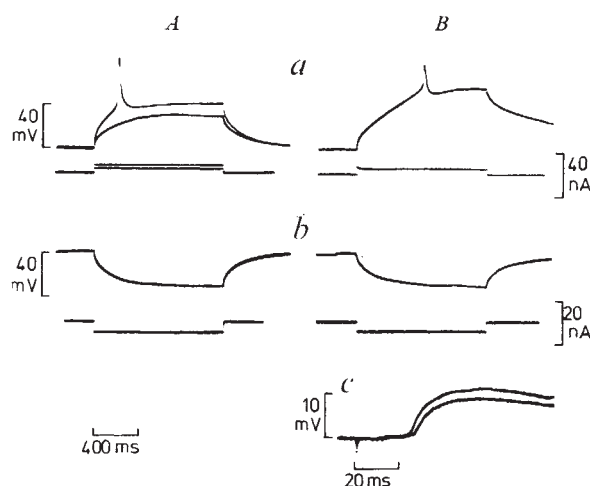
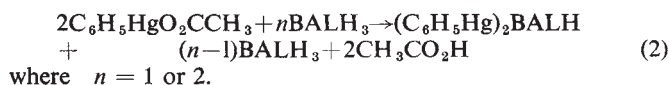
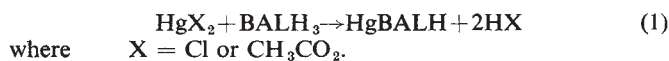


Fig. 1 Action potentials (a) and anelectrotonic potentials (b) recorded from a denervated (A) and an innervated (B) slow fibre 9 days after cutting spinal nerve IX. Upper trace, membrane potential, lower traces, membrane currents. Note large membrane time constant typical of slow fibres. c, End-plate potentials elicited in innervated slow fibre by stimulation of spinal nerve VIII at two points 10 mm apart; from the difference in latencies a conduction velocity of 4.4 m s^{-1} was calculated for the motor axon. Both fibres from same muscle. Note also the presence of miniature end-plate potentials in trace b of fibre B.

of BALH_3 (ref. 8), this distribution is undesirable in cases of chronic mercury poisoning where the critical organ is the brain, in which it is metabolised more slowly than in most organs^{10,11}.

An understanding of the chemistry involved in the interaction of BALH_3 with mercury compounds, and of the properties of any complexes formed, is necessary to assess these observations. Isolation of $\text{HgBALH}^{1,12}$ has been reported and evidence for the formation of $\text{Hg}(\text{BALH}_2)^{2-}$ (ref. 1), $(\text{C}_6\text{H}_5\text{Hg})_2\text{BALH}$ (ref. 12) and $(\text{RHg})_n\text{BALH}_{3-n}$ ($n = 1$ (ref. 13), 2 (ref. 12); $\text{R} = \text{CH}_2\text{CH}(\text{OCH}_3)\text{CH}_2\text{R}'$) has been documented.

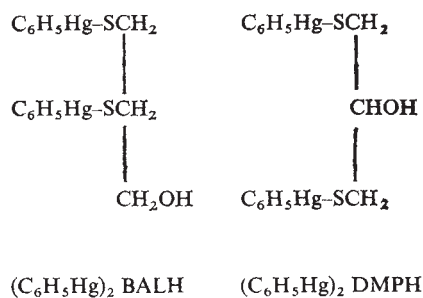
We have found that reaction of mercuric salts and phenylmercuric acetate with BALH_3 in water leads to precipitation of HgBALH and $(\text{C}_6\text{H}_5\text{Hg})_2\text{BALH}$, respectively:



To assist interpretation of physical and spectroscopic data for the complexes (Table 1) we prepared the analogous 1,3-dimercaptopropanol(DMPH₃) complexes.

All the complexes are insoluble in water and common organic solvents. Except for HgBALH they dissolve in the coordinating solvents pyridine and dimethylsulphoxide; this may indicate polymeric structures or strong intermolecular forces in the solid state. Assignment of $\nu(\text{Hg-S})$ and $\nu(\text{O-H})$ in infrared spectra of the complexes (Table 1) establishes the basic mode of attachment of mercury to BALH and the loss of two thiol protons on complex formation. Assignment of a coordination number for mercury is, however, not possible since $\nu(\text{Hg-S})$ does not vary greatly with coordination number in related compounds, for example, two-coordinate mercury in $\text{Hg}(\text{SC}_2\text{H}_5)_2$ (ref. 14) has $\nu(\text{Hg-S})$ at 330 cm^{-1} , and four-coordinate mercury in *bis* (O-ethylthioacetothioacetato) mercury has $\nu(\text{Hg-S})$ at 362 cm^{-1} (ref. 15).

HgDMPH is associated in pyridine (Table 1), consistent with a polymeric or oligomeric structure in the solid state, and HgBALH presumably adopts a similar structure. Molecular weight data indicate monomeric structures for the phenylmercury derivatives in pyridine:



Since a variety of 'solvent' environments, for example, aqueous and lipid, are present in organisms, the stability of $(\text{C}_6\text{H}_5\text{Hg})_2\text{BALH}$ in different solvents is of particular interest. In aqueous suspension, $(\text{C}_6\text{H}_5\text{Hg})_2\text{BALH}$ is stable with an excess of BALH_3 since it can be prepared with $n = 2$ (equation (2)); and the possible decomposition products expected, $(\text{C}_6\text{H}_5)_2\text{Hg}$, HgBALH or benzene (gas liquid chromatography), could not be detected. Although stable in pyridine and dimethylsulphoxide (Table 1), addition of acetone to a pyridine solution results in precipitation of HgBALH and formation of diphenylmercury. When $(\text{C}_6\text{H}_5\text{Hg})_2\text{BALH}$ is stirred as a slurry in benzene for 19 h, the insoluble residue contains $(\text{C}_6\text{H}_5\text{Hg})_2\text{BALH}$ and HgBALH ; $(\text{C}_6\text{H}_5)_2\text{Hg}$ can be isolated from the solution. Similarly, reaction of phenylmercuric acetate with BALH_3 (2:1) in methanol for 2 h gives a mixture

Table 1 Characterisation of 2,3-dimercaptopropanol (BALH_3) and 1,3-dimercaptopropanol (DMPH_3) complexes*

Complex	Infrared (cm^{-1})†		Molecular weight‡	
	$\nu(\text{Hg-S})$	$\nu(\text{O-H})$	Observed	Calculated for monomer
HgBALH	350	3,325	Insoluble	323
$(\text{C}_6\text{H}_5\text{Hg})_2\text{BALH}$	335	3,330	664 ± 15	678
HgDMPH	353	3,330	500 ± 67 §	323
$(\text{C}_6\text{H}_5\text{Hg})_2\text{DMPH}$	343	3,320	653 ± 43	678
$(\text{C}_6\text{H}_5\text{Hg})_2\text{DMPH} \cdot \text{C}_5\text{H}_5\text{N} $	343	—	750 ± 34	757
$\text{C}_6\text{H}_5\text{HgSCH}_2\text{CH}_2\text{OH} $	343	3,230	364	355
$\text{Hg}(\text{SCH}_2\text{CH}_2)_2 $	330	—	330	323

* All complexes have satisfactory elemental analyses (C, H, Hg, S) and, except for insoluble HgBALH , have satisfactory ^1H NMR spectra in both d^5 -pyridine and d^6 -DMSO.

† Nujol mulls. All absorptions listed are broad. $\nu(\text{S-H})$ is absent from all spectra, indicating loss of two protons on formation of each complex.

‡ In pyridine at 60°C determined by vapour pressure osmometry. Where several determinations were obtained the range is given. Since pyridine is a non-ideal solvent, accurate molecular weights are not expected. However, complex formation with pyridine (see for example ¶ below) as one form of non-ideal behaviour is not expected to affect the accuracy of determinations, since the colligative property determined is the number of molecules formed in solution per solid complex added; and this will not be altered by interaction with the solvent which is present in vast excess.

§ These results are intermediate between monomer and dimer, indicating the presence of a monomer-oligomer(s) (most likely dimer) equilibrium. In addition NMR spectra have a very broad methylene resonance, suggesting more than one methylene environment.

¶ $(\text{C}_6\text{H}_5\text{Hg})_2\text{DMPH}$ forms a crystalline 1:1 adduct with pyridine. An unaltered $\nu(\text{Hg-S})$ and lowered $\nu(\text{O-H})$ (very broad absorption beneath $\nu(\text{C-H})$ bands at $2,800$ – $3,200\text{ cm}^{-1}$) indicate that pyridine is hydrogen-bonded to the hydroxyl group, rather than coordinated to mercury. With the hydroxyl group deuterated $\nu(\text{O-D})$ occurs at $2,200\text{ cm}^{-1}$. The calculated molecular weight is that expected for dissolution of a 1:1 mixture of $(\text{C}_6\text{H}_5\text{Hg})_2\text{DMPH}$ and pyridine in pyridine.

|| Included for comparison.

of $(\text{C}_6\text{H}_5\text{Hg})_2\text{BALH}$, HgBALH and $(\text{C}_6\text{H}_5)_2\text{Hg}$. Thus, with some solvents, $(\text{C}_6\text{H}_5\text{Hg})_2\text{BALH}$ decomposes according to



The formation of diphenylmercury had not previously been suspected and may be an important factor in the distribution of mercury when BALH_3 is administered with phenylmercuric acetate. Diphenylmercury is less polar than Hg^{2+} and $\text{C}_6\text{H}_5\text{Hg}^+$, does not form complexes with neutral ligands¹⁶ and does not react with thiols^{17,18} or BALH_3 at ambient temperature. The toxicity of diphenylmercury does not seem to have been investigated, except for a brief report¹⁹ that diphenylmercury in 'scarcely detectable' concentration, formed by degradation of phenylmercuric acetate (formerly contained in derelict steel drums), was sufficiently toxic to kill fish within a few hours in the Boone Reservoir, Tennessee Valley.

These preliminary results indicate the complexity of antidote action and the need for caution in application of BALH_3 as an antidote for chronic phenylmercury poisoning. This is particularly true before degradation to inorganic mercury, which is substantially complete after 1 d in laboratory animals²⁰⁻²². This study is being extended to include alkylmercury compounds.

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Effect of unsaturated fatty acids on the lipid composition of bacteriophage PM2

A LIPID-containing bacteriophage, PM2, provides a simple model to investigate assembly of lipids and proteins into a membrane. The phospholipid in the virion, which is arranged in the form of a bilayer between nucleoprotein core and outer shell¹, has a very different composition from that of the host cell, a marine bacterium, *Pseudomonas* BAL-31 (ref. 2). One-third of the viral lipids are derived from lipids synthesised before infection and two-thirds from lipids synthesised during viral replication³.

We have studied the variation in viral fatty acids and phospholipids when the virus was grown in an auxotroph

requiring unsaturated fatty acids, *Pseudomonas* BAL-31, strain UFA (ref. 4). The fatty acid composition in host cells and virions varied in a similar manner in media supplemented with different fatty acids, whereas the phospholipid composition of the host cell was almost constant; that of the virion varied depending on the fatty acids used in the media.

Effects of exogenous unsaturated fatty acids on the viral lipid composition were determined using *Pseudomonas* BAL-31, UFA as host. The fatty acid composition in host cells and virions grown in media supplemented with unsaturated fatty acids is shown in Table 1. In media supplemented with palmitoleic (*cis*-16:1) and oleic (*cis*-18:1) acids, the fatty acid composition of phospholipids in virions and host cells seemed to be identical, within experimental error. In medium supplemented with palmitelaidic acid (*trans*-16:1), the amount of palmitic acid (16:0) in virions was larger than that in host cells and the amount of 16:1 in virions was smaller. Virions grown in media supplemented with *cis*-16:1 and *trans*-16:1 had nearly homogenous fatty acid compositions, 16:1 accounting for 89% and 74% of the total fatty acids, respectively. In general, the fatty acid composition in virions reflects that in the host cells and thus can be altered by variation in the exogenous fatty acid.

The phospholipid composition in host cells and virions grown in media supplemented with unsaturated fatty acids is shown in Table 2. In all media except that supplemented with *trans*-16:1, the major phospholipid in virions was phosphatidylglycerol (PG). In medium with *trans*-16:1, phosphatidylethanolamine (PE) became the dominant viral phospholipid. The ratio of PG to PE in viral lipids varied with the input fatty acid: in virions grown on *Pseudomonas* BAL-31 (wild type) in a medium without added fatty acid it was about 1.5; in virions grown on strain UFA in media supplemented with *cis*-16:1 or *cis*-18:1 it was about 1.2; and in virions grown on strain UFA in *trans*-16:1 medium about 0.8. In earlier studies^{2,5} the PG/PE ratio in virus grown on wild type cells in a medium containing the same salts as used above, plus arginine, proline and glucose, was about 2.4. This discrepancy may be the result of differences in the medium used. In contrast to the situation with fatty acids and phospholipids, virions purified from strain UFA grown in the presence of different fatty acids had the same pattern of polypeptides (using SDS-polyacrylamide gel electrophoresis) as those prepared from wild type *Pseudomonas* BAL-31.

Table 1 Fatty acid composition of phospholipids in *Pseudomonas* BAL-31/UFA and PM2 grown in media supplemented with unsaturated fatty acids

Fatty acids in phospholipid	Unsaturated fatty acid added to growth medium					
	Palmitoleate (<i>cis</i> -16:1)		Palmitelaidate (<i>trans</i> -16:1)		Oleate (<i>cis</i> -18:1)	
	UFA	PM2	UFA	PM2	UFA	PM2
14:0	—	—	trace	trace	0.5	0.4
14:1	0.6	2.0	10.7	10.0	1.4	1.9
16:0	3.0	4.7	0.7	7.6	7.2	4.7
16:1	88.8	89.0	86.1	74.2	57.2	58.8
18:0	0.6	0.3	0.2	3.4	0.9	0.7
18:1	7.0	4.0	2.3	5.0	32.8	33.5
Saturated	3.6	5.0	0.9	11.0	8.6	5.8
Unsaturated	96.4	95.0	99.1	89.0	91.4	94.2
Unknown	trace	trace	trace	trace	trace	—

For the isolation of fatty acid auxotrophs, *Pseudomonas* BAL-31 was grown at 25°C in AMS salts¹² supplemented with 2 g l⁻¹ of the amino-acid mixture containing 0.1 g l⁻¹ of twenty amino acids (AA medium) and 0.01% oleic acid dissolved in 0.4% bovine serum albumin containing less than 0.05% fatty acids. After ultraviolet irradiation to the 0.5% survival level, fatty acid auxotrophs were isolated using penicillin enrichment in AA medium and replica plating on selective media. A culture (25 ml) of *Pseudomonas* BAL-31 fatty acid auxotroph strain UFA grown in the presence of different unsaturated fatty acids was infected (multiplicity of infection 10) with PM2 which had been grown on wild type cells. This lysate was used to infect 100 ml cultures, which were used in turn for 1 l of culture. Virus was purified from 1 l lysate 3 h after infection⁶. The purity of each virus preparation was controlled by SDS-polyacrylamide gel electrophoresis¹³. Lipids were extracted from purified virus and from host cells which were collected just before infection³. Phospholipids were then isolated on silica gel thin layer plates (Merck, *F₂₅₄*) using diethylether-benzene-ethanol-acetic acid (40:50:2:0.2, v/v). The area corresponding to the phospholipids was made visible with iodine vapour, then scraped and eluted with chloroform-methanol (2:1, v/v). Phospholipid was trans-methylated with BF₃-methanol (14%, w/v; Applied Science Laboratories). Methyl esters were analysed on a Packard model 7620A gas chromatograph with 10% EGSSX on Chromosorb W and 3% SE30 on Gas Chrom Q. Methyl esters were identified by comparison of their retention times with those from a KD ester mixture (Applied Science Laboratories). Results are expressed as percentage composition.

Table 2 Phospholipid composition in *Pseudomonas* BAL-31/UFA and PM2 grown in media supplemented with unsaturated fatty acids

Phospholipid	Unsaturated fatty acid added to growth medium						None	
	Palmitoleate (<i>cis</i> -16:1)		Palmitelaidate (<i>trans</i> -16:1)		Oleate (<i>cis</i> -18:1)			
	UFA	PM2	UFA	PM2	UFA	PM2	BAL-31	PM2
Compound X	0.2	1.8	3.8	6.9	0.1	0.8	0.1	0.6
	0.1	1.0	2.7	1.2	0.1	1.0	0.4	0.6
Phosphatidyl-ethanolamine	78.8	41.7	82.3	49.6	80.8	47.0	75.8	39.5
Phosphatidyl-glycerol	85.6	43.7	84.8	53.8	89.1	41.2	80.5	38.4
Lysophosphatidyl-ethanolamine	18.9	53.3	12.1	41.1	17.4	50.0	22.0	58.2
Others	13.2	52.5	11.0	42.0	10.2	55.8	17.9	59.4
	2.0	1.7	1.7	1.2	1.3	1.4	1.6	0.9
	0.4	1.8	1.0	2.1	0.4	1.4	0.6	1.0
	0.1	1.6	0.1	1.2	0.4	1.1	0.5	0.9
	0.6	1.1	0.6	0.9	0.2	1.0	0.6	0.7
Total radioactivity (c.p.m.)	97,019	102,381	83,500	73,474	74,491	65,926	65,002	99,916
	82,910	28,604	65,143	28,458	61,135	25,686	59,417	57,324
Ratio PG/PE	0.24	1.28	0.15	0.83	0.22	1.06	0.29	1.47
	0.15	1.20	0.13	0.78	0.12	1.36	0.22	1.55

Pseudomonas BAL-31 and strain UFA were grown at 25° C in AA medium with 0.4% bovine serum albumin and 0.01% essential fatty acid. The culture (25 ml) was lysed by PM2 grown on wild type cells. This lysate was used in turn for lysing 100 ml culture containing 3 μ Ci ml⁻¹ ³²P. Virus was purified along with carrier virus grown on wild type cells and its purity was controlled by SDS-polyacrylamide gel electrophoresis, as described in Table 1. Lipids were extracted from virus and host cells, which were withdrawn just before infection as described in Table 1. Lipids were analysed on silica gel thin layer plates (Merck, F_{254}) using chloroform-methanol-water (65:25:4, v/v). Each class of phospholipids was detected by exposure to Ilford X-ray film, scraped into vials and counted for radioactivity². Results are expressed as percentage composition.

There are four viral proteins: I and II form spikes and outer shell, respectively; III forms the inner shell of the nucleocapsid; while IV is associated with viral DNA (ref. 6). Proteins I, III and IV have isoelectric points at pH 6.2, 5.8 and 5.5, respectively, whereas the isoelectric point of protein II is at pH 12.3. Thus protein II is strongly positively charged at pH 7.0 (refs 7 and 8). The electrostatic interaction between protein II and PG has been suggested as a major factor controlling phospholipid composition and also stabilising the bilayer structure of the completed particle^{7,8}. The PG/PE ratio would remain constant, provided that the fatty acids do not affect the charge distribution and that long range forces play only a minor role. In addition to electrostatic forces, there is evidence for hydrophobic interactions between the bilayer and the inner protein shell as protein III behaves as a proteolipid^{8,9}, and some viral protein(s) seems to penetrate the lipid bilayer and may also participate in lipid-protein interactions.

The physical properties of phospholipids containing different hydrocarbon side chains have been studied using monolayer techniques¹⁰. *Trans*-unsaturated fatty acids have properties intermediate between *cis*-unsaturated fatty acids and saturated fatty acids, forming more condensed films than *cis*-unsaturated fatty acids. Virions grown on strain UFA in the presence of exogenous *trans*-16:1 should therefore have the most condensed lipid bilayer of those reported here, which should lead to a stronger hydrophobic interaction between proteins and lipids within the membrane structure and may thereby overcome the electrostatic interaction. Furthermore, if we compare the known mobility of PE and PG (ref. 11) with the phospholipid composition of virions containing *trans*-16:1, the average fluidity must have decreased. Thus the change in phospholipid composition can hardly be due to a tendency to maintain average fluidity. Although the phospholipid composition in virions varies according to the fatty acid used in the medium, such variations are controlled within narrow limits. Thus both hydrophobic and electrostatic interactions between lipids and proteins probably assist in maintaining viral integrity.

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The double helix of tropomyosin

THE tropomyosin molecule consists of two α helices, which form a coiled-coil a little over 400 Å long¹. Besides forming crystals, tropomyosin can be precipitated with divalent cations to produce needle shaped aggregates (showing a variety of patterns of cross striations in the electron microscope²) for which the detailed molecular packings have not been established. At low resolution, however, the α helices may be regarded as smooth rods. The structure of such aggregates could depend, to some extent, on considerations of closest packing of the rods. This possibility seems to have been first considered by Rudall³ in his discussion of the crystal structure of the α protein from Mantis egg case, where he showed that double helices may be close-packed to form sheets by staggering each double helix by one quarter of the pitch with respect to the next. I report here that the idea of a quarter stagger between tropomyosin molecules, in conjunction with certain electron microscope results, leads to a value for the pitch of the tropomyosin helix.

Figure 1a shows an electron micrograph of vertebrate tropomyosin precipitated⁴ with CaCl₂ at pH 8, displaying sets

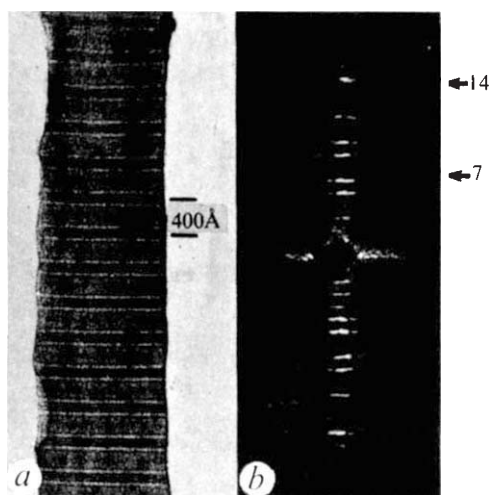


Fig. 1 *a*, Rabbit striated muscle tropomyosin precipitated with 20mM CaCl_2 at pH 8. *b*, Optical diffraction pattern of (*a*).

of transverse striations repeating axially every 400 Å. A striking feature of the optical diffraction pattern of this form is a prominent 14th order of the 400 Å repeat while the 7th order is weak or absent (Fig. 1*b*). This suggests that there is a component in the structure which repeats every 57 Å but which is staggered in alternate units by one half of this distance. This suggestion has also been made by Parry and Squire⁵ who noticed that the electron micrographs of this form of tropomyosin show subperiods of about 28 Å and that "the separation of bands related by twofold symmetry is always an odd multiple of 28 Å".

These observations lead to the hypothesis that the pitch of the double helix in tropomyosin is 114 Å, in which case the repeat distance in the molecule would be 57 Å (the half-pitch); close packing would halve this to give a repeat in the sheets of 28.5 Å. The sheets could then superimpose, forming the observed structures. (The various ways in which such sheets can pack together to form three dimensional structures will be discussed elsewhere.) Accumulation of negative stain between the helices would then produce the subperiods observed with the electron microscope.

In the living muscle, tropomyosin is associated with actin which consists of two strands of subunits repeating along a helix with pitch of about 700 Å. Tropomyosin molecules lie in the two grooves, one on each side of the actin helix, and each molecule appears to interact with one strand. The vertical distance between the subunits of actin is about 55 Å. The distance along the helix between the subunits at the radius at which tropomyosin lies, is, however, close to 57 Å. (These findings have been summed up by Huxley⁶.) Thus, if the pitch of the tropomyosin double helix were 114 Å, each actin subunit would be in a position to interact with one half-turn of this helix. In this case, the chemical sequence of tropomyosin would be expected to display a similar periodicity, since each half-turn would have a similar chemical function to perform. An indication of such periodicity has recently been pointed out by Parry⁷ for the partial sequence of tropomyosin published by Hodges *et al.*⁸. Parry observed a pseudo-repeat of about 29 Å in the various types of residue groupings along the chain, so that each successive pair of these groups would lie opposite one actin subunit. Only if the pitch of the tropomyosin double helix were commensurate with the actin periodicity, however, could the repeating groups face towards the actin subunits to present to each a similar aspect.

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Reaction of ribonucleosides and deoxynucleosides with copper(II) acetate

BERGER *et al.* reported¹ that ribonucleosides and deoxynucleosides can be differentiated by their effects on the optical spectra of copper(II) acetate in dimethylsulphoxide (DMSO). They suggested that the 2' and 3' oxygen atoms in ribonucleosides bridge pairs of copper atoms, forming 1 : 1 copper(II) acetate/ribonucleoside complexes¹. We have examined the species in such solutions by EPR. Optical spectra are not ideal as the bands are broad, and components of mixtures cannot readily be resolved. EPR spectra of spin-coupled ($S = 1$) copper(II) dimers differ appreciably, however, from those of monomeric ($S = \frac{1}{2}$) compounds. Moreover, they are sensitive to the nature of the bridges²⁻⁵ and to the symmetry about the Cu-Cu axis. Our conclusions from these studies differ from those obtained previously¹.

The X-band spectra of frozen DMSO solutions of copper(II) acetate alone and with various ribonucleosides and deoxynucleosides are shown in Fig. 1. As in previous work¹ fresh solutions were used (10^{-2} M in copper(II) acetate). The $S = 1$ bands at about 40, 460 and 590 mtesla (mT) (Fig. 1*a*) confirm that copper(II) acetate is dimeric in DMSO. The spectrum of a 1 : 1 copper(II) acetate dimer/uridine solution (Fig. 1*b*) shows that uridine cleaves the dimer forming a large amount of a monomeric copper(II) species, with a strong signal at about 300 mT. There is no evidence for a dimer containing ribonucleoside bridges, which would give new $S = 1$ bands. Moreover, the presence of both acetate and ribonucleoside bridges would result in $E \neq 0$ and a split H_{xy} band⁶. Instead, the residual $S = 1$ spectrum is that of copper(II) acetate.

The spectrum of copper(II) acetate and deoxyuridine (Fig. 1*c*) shows that, in contrast to conclusions drawn¹ from optical spectra, the deoxynucleoside also cleaves the dimer, but to a lesser extent than uridine under the same experimental conditions. Deoxythymidine gave similar results. Both cytidine and deoxycytidine cause appreciable rupture of the dimer (Fig. 1*d*). Deoxycytidine is more effective in this respect than deoxyuridine or deoxythymidine. The spectrum with cytidine differs significantly from that with uridine in the $g_{\parallel}$ region of the monomer. Whereas the monomers formed by uridine, deoxyuridine and deoxythymidine each show one set of $g_{\parallel}$ bands (Fig. 1*e*), with cytidine (Fig. 1*f*) there are two sets of $g_{\parallel}$ components (A , $g_{\parallel}$ 2.304, $A_{\parallel}$ 15 mT; B , $g_{\parallel}$ 2.249, $A_{\parallel}$ 17.5 mT) indicating the presence of two monomeric species.

These results suggest that differentiation between ribonucleosides and deoxynucleosides by copper(II) acetate does not result from bridging by the 2' and 3' OH groups, but from the relative ease with which the dimeric structure is broken. Assuming complete protonation of N(3) in deoxyuridine and uridine, the major donor sites will be the sugar hydroxyl groups. Coordination of the 2' or 3' OH group of uridine to the terminal position of copper(II) acetate favours formation of a chelate ring by displacement of a neighbouring carboxylate oxygen by the other OH group of the ribonucleoside. Rupture of one acetate bridge would be followed by complete break-up of the dimer. As this mechanism is not available to deoxyuridine or deoxythymidine a differentiation between ribonucleoside and deoxynucleoside becomes possible.

We have observed, however, that deoxynucleosides also cause some rupture of the dimer. A possible reason is suggested by the results obtained with cytidine and deoxycytidine. Coordination of the non-protonated nitrogen, N(3), in cytidine

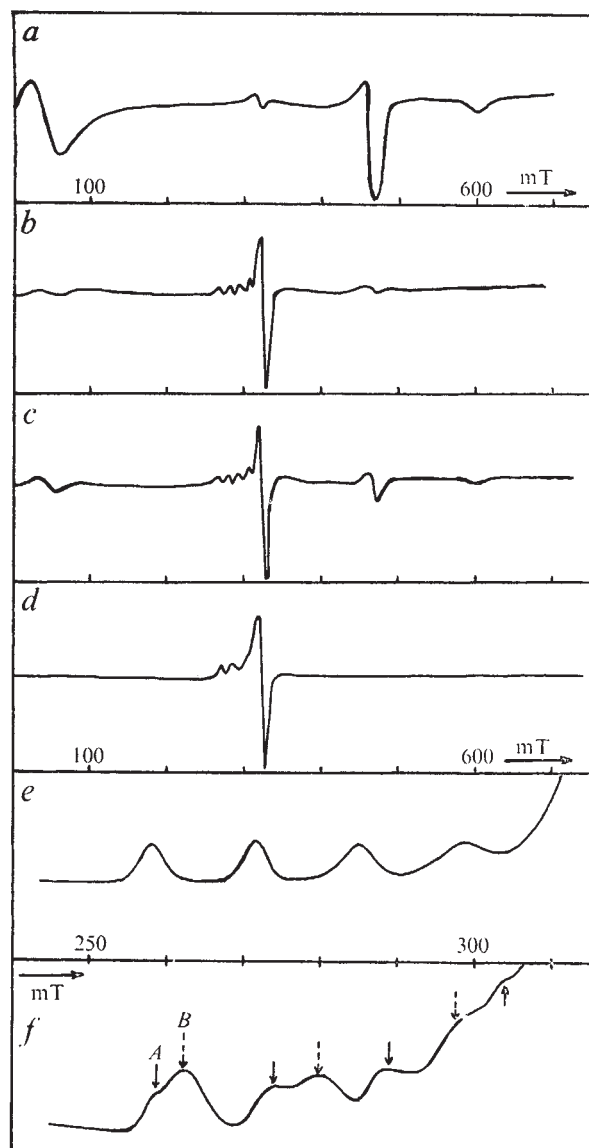


Fig. 1 X-band EPR spectra of frozen DMSO solutions of *a*, copper(II) acetate; *b*, copper(II) acetate and uridine (1 dimer: 1 nucleoside); *c*, copper(II) acetate and deoxyuridine (1 dimer: 1 nucleoside); *d*, copper(II) acetate and cytidine (1 dimer: 1 nucleoside); *e*, as *b*, but showing the $g_{\parallel}$ bands of the $S=\frac{1}{2}$ spectrum in more detail; *f*, as *d*, but showing the $g_{\parallel}$ bands of the $S=\frac{1}{2}$ spectrum in more detail.

and deoxycytidine to the terminal position in copper(II) acetate would bring the oxygen on C(2) and the amine group on C(4) close to acetate oxygen atoms and thus destabilise the dimer. With deoxycytidine this would be the main mechanism as there is no 2' OH group. With cytidine, however, either mechanism, that is, chelation by 2' and 3' OH groups or coordination by N(3), could operate. This would account for the two monomeric species observed. In polar solvents the N-H proton in deoxyuridine and deoxythymidine could undergo keto-enol tautomerism. Copper(II) acetate would displace this equilibrium towards the enol form by coordinating the non-protonated N(3) atoms. This would enable deoxyuridine and deoxythymidine to interact with copper(II) acetate, though to a smaller extent than deoxycytidine, as observed.

We conclude that copper(II) acetate does not 'recognise' the difference between ribonucleosides and deoxynucleosides by serving as a template for ribonucleoside bridges. Instead, the factors involve competing influences of the various co-

ordinating groups present, and in a broader biological context these influences will vary with the metal ion^{7,8}.

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Statistical significance of DNA sequence symmetries

SOME regions of DNA involved in regulatory or catalytic interactions with proteins have sites with twofold rotation symmetry in the primary nucleotide sequence¹⁻³. It would be worthwhile to determine whether certain of these symmetries occur so rarely by chance that they probably have a function, for example, as recognition sites for symmetric protein complexes. Likewise, it would be useful to be able to dismiss other apparent symmetries as probable statistical accidents of no functional importance. The following quantitative method can be used to evaluate the statistical significance of hyphenated (that is, incomplete) symmetries, where a more intuitive approach may be misleading. Since the probability of random occurrence of a given subsequence increases in almost direct proportion to the length of the sequence considered, a rigorous approach will become increasingly important in the evaluation of the significance of subsequence anomalies occurring in the much longer DNA sequences that will be determined in the future.

Rotation symmetry is said to exist in a DNA site if rotation about a twofold axis at the centre of the site and perpendicular to the DNA helix axis does not change the structure of the site. Various rotationally symmetric sites in bacteriophage λ DNA in the region of the joined cohesive ends (*cos*) and the region of repressor binding are indicated in Fig. 1. These symmetries are imperfect, and not all positions in the sites are invariant under rotation. The base pairs in these sites to be evaluated for symmetry have been marked *s*, *p* or *h* to designate whether under the rotation symmetry transformation they remain invariant (*s*), change but retain the same purine-pyrimidine orientation (*p*), or do neither of the above (*h*). The λ *cos* site designated in Fig. 1*a* has five perfect symmetries (*s*), five purine-pyrimidine symmetries (*p*), and one hyphen (*h*). A site with this extent of symmetry will be designated by the notation ($5s_5p_1h$). Obviously, there is some probability that this symmetry could have arisen entirely by statistical accident. In a random sequence of 50% GC base composition, the *a priori* probability of an isolated occurrence of an *s* is 1/4, of a *p* is 1/4, and of an *h* is 1/2, so the *a priori* probability of the symmetry ($5s_5p_1h$) in a sequence of length 2 ($S+P+H$) is

$$\frac{(S+P+H)! (1/4)^S (1/4)^P (1/2)^H}{S! P! H!}$$



Fig. 1 Symmetrical sites in certain regions of λ DNA. Bracketing lines indicate the two halves of a site symmetrical about a twofold rotation axis positioned at the symbol showing a dot within a circle. The symbol | designates the positions in λ cos at which single strand nicks must be introduced to generate cohesive ends. Other symbols are explained in the text. a, λ cos region (region of joined cohesive ends)³⁻⁵, 5,5_p1_h symmetry site; b, region of λ repressor binding¹, 8,3_p1_h symmetry site; c, region of λ repressor binding¹, 7,3_p3_h symmetry site; d, region of λ repressor binding¹, 7,6_p2_h symmetry site.

The factorial coefficient arises because the order in which the various symmetries would occur in the site was not specified beforehand, so all significant permutations must be counted.

When a region of DNA is examined for sequence symmetries, the intention is usually to note all sites with sufficient symmetry to be of interest, rather than only sites of one specified degree of symmetry. Hence a satisfactory criterion for regarding an observed sequence to be exceptionally symmetrical is provided, not by the probability of occurrence of the symmetry, but rather by the probability that a random sequence of equal length would have an equal or greater symmetry.

The *a priori* probability, E_p , that at least M out of N positions in a site are either purine-pyrimidine symmetrical (p) or perfectly symmetrical (s) is

$$E_p = \sum_{i=M}^N \sum_{j=0}^i \frac{N! (1/4)^j (1/4)^{i-j} (1/2)^{N-i}}{j! (i-j)! (N-i)!}$$

$$= (1/2)^N \sum_{i=M}^N \sum_{j=0}^i \frac{N! (1/2)^i}{j! (i-j)! (N-i)!}$$

which is obtained by summing the individual probabilities of all such sites. Similarly, the *a priori* probability, E_s , that at least M positions in a site of length N are purine-pyrimidine symmetrical or perfectly symmetrical, and of these at least R positions are perfectly symmetrical is

$$E_s = (1/2)^N \sum_{i=M}^N \sum_{j=R}^i \frac{N! (1/2)^i}{i! (i-j)! (N-i)!}$$

The latter quantity, E_s , is of greater interest because it can be assumed that perfect symmetry will be more important than purine-pyrimidine orientation symmetry in most DNA recognition schemes, but the expectation of the symmetry can be shown to increase to E_p when the base composition of the region investigated is biased, for independent reasons, very heavily towards either GC or AT. For evaluation of an ($S_p P_p H_h$) symmetry, the appropriate values of M , R and N in the above expressions are given by $M = S+P$, $R = S$, $N = S+P+H$. The expressions are most conveniently evaluated by computer.

The values of E_p and E_s for the sites shown in Fig. 1 are tabulated in columns 3 and 4 of Table 1. These values reveal

Table 1 Symmetry and statistical significance of sites shown in Fig. 1

Site illustrated	Rotation symmetry type	E_p : per-site probability of greater or equal purine-pyrimidine symmetry	E_s : per-site probability of greater or equal total symmetry	No. of sites of similar length in region considered	Expected frequency of sites of greater or equal total symmetry in region considered
Fig. 1a	5,5 _p 1 _h	5.86×10^{-3} = 1/171	3.70×10^{-3} = 1/270	—	—
Fig. 1b	8,3 _p 1 _h	3.17×10^{-3} = 1/315	3.79×10^{-4} = 1/2637	19	0.007
Fig. 1c	7,3 _p 3 _h	4.61×10^{-3} = 1/22	9.29×10^{-3} = 1/108	19	0.177
Fig. 1d	7,6 _p 2 _h	3.69×10^{-3} = 1/270	1.90×10^{-3} = 1/526	11	0.021

Column 2 indicates the symmetry type of the site according to the notation introduced in the text. Columns 3 and 4 give the rarity of occurrence of at least this degree of symmetry in a single site. Column 5 gives the number of such sites that can be tested for symmetry in the region under consideration, and the value in column 6, the product of values in columns 4 and 5, gives the expected frequency of occurrence of a site showing at least this degree of symmetry in the whole region under consideration.

that only about one sequence in 200 of length 22 would show at least the $(5_s, 5_p, 1_h)$ symmetry observed in the λ *cos* site. It would seem likely that this symmetry reflects some underlying mechanism. The significances of the symmetries at sites in the repressor-binding region of λ DNA vary considerably. When considered alone, these sites all appear to be quite rare, but this is misleading. If λ cohesive ends are generated by a symmetrical recognition-catalysis complex, the symmetry axis should, as it does, lie equidistant from the two sites at which nicks are to be introduced (Fig. 1a). There is no such *a priori* basis for site selection, however, in the case of the λ repressor-binding region: symmetries were considered wherever found. If the symmetry axis lies either on a base pair (as in Fig. 1b) or between two base pairs (as in Fig. 1c), then the number of sites comprised of two sequences of length N separated by zero or one base pair which can be tested for symmetry in a total sequence of length L is given by $2L-4N+1$. When each per-site expectation of symmetry is multiplied by the number of available sites, the results (Table 1, column 6) reveal that sites having at least $(8_s, 3_p, 1_h)$ symmetry, or $(7_s, 6_p, 2_h)$ symmetry, would be expected to occur in regions of 35 base pairs with a frequency of 0.7%, or 2%, respectively, but that sites having at least $(7_s, 3_p, 3_h)$ symmetry would be expected to occur with an 18% frequency. Hence the symmetries shown in Fig. 1b and d would seem likely to be functionally significant, but the symmetry of Fig. 1c could well be due to chance.

It can be shown that in a random DNA sequence the occurrence of symmetry about one rotation axis does not affect the probability of symmetry about a second, distinct axis (my unpublished work). Two sites symmetrical about different axes can therefore be considered independently, even if the sites overlap. This independence does not generally hold for more than two overlapping sites.

The lack of statistical significance in the observation of a symmetry does not necessarily imply that the symmetry is not functionally significant. Trial calculations reveal that it may be impossible to distinguish statistically certain functional symmetries from spurious symmetries unless the region under consideration can be restricted independently to considerably fewer than 100 base pairs.

This analysis determines the position of an observed symmetrical site in the symmetry-ordered distribution of only those sites which are of the same length. Although this comparison is limited, it provides an easily-computed estimate of the rarity of the symmetry. A similar approach could be used in the statistical evaluation of other types of sequence anomalies such as purine-pyrimidine orientation bias.

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Single-stranded regions in DNA of old mice

INVESTIGATIONS have shown that senescence is accompanied by molecular changes in the structure of the eukaryotic genome. Some of these changes have been reported to result in the loss of some genetic material in the brain cells of ageing beagles¹. Furthermore, the molecular weight of rat liver DNA decreases as a function of senescence². If such changes occurred on a large scale, the resulting genetic damage could affect severely some of the crucial gene functions of an organism. We are

studying the physical state of the DNA of senescing mice, and report here the accumulation of an increasing amount of single-stranded regions in the DNA with age.

DNA was isolated from the livers of 1, 6, 15, 20, 25, and 30-month-old CBF₁ mice (Charles River Breeding Laboratories). Single-strand-specific nuclease *S*₁ was used to investigate the presence of single-stranded regions in the native DNA of mice of different ages. Figure 1 shows the absence of nuclease *S*₁ sensitivity in the DNA of mice aged 1-15 months. That of mice 20 months of age or older was increasingly sensitive to the enzyme, which digested 14-25% of it. We cannot rule out the possibility that this sensitivity began before the mice were 20 months old, since we did not test the DNA of 15-20-month-old mice. These results are based on three experiments in each of which the DNA from different animals of the same age group were used.

There are already two sets of indirect evidence for single-stranded regions in the DNA of ageing animals. Samis *et al.*³ reported increased incorporation of ³H-thymidine into the nuclear DNA of senescent rats in the absence of a corresponding increase in mitotic index. This implies that the thymidine, after conversion to dTTP, was used in the repair of nuclear DNA. Calf thymus DNA polymerase has been shown to catalyse a greater incorporation of DNA precursors into the nuclei of old mouse neurones, astrocytes, Kupffer cells and heart muscle fibres⁴. This reaction is known to require single-stranded regions of primer DNA along which the complementary strand can be synthesised⁵. Presumably these single-stranded regions of the DNA from senescent mice were hydrolysed by nuclease *S*₁ in our experiments.

As we do not know the ultimate fate of the nuclease *S*₁-sensitive regions in the DNA of old mouse liver, we suggest that

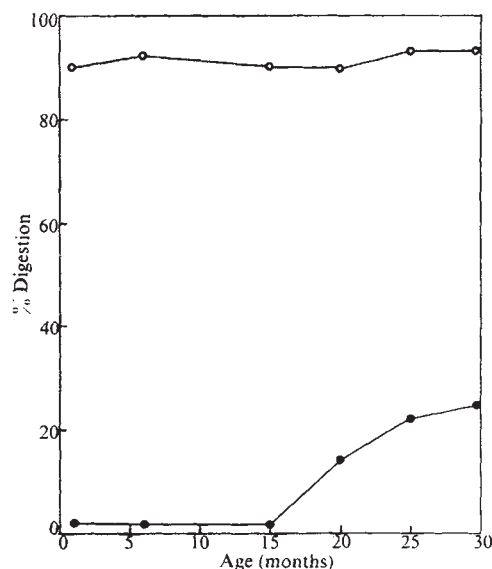


Fig. 1 Nuclease *S*₁ digestion of 1-30-month-old mouse liver DNA. DNA was prepared essentially by the method of Marmur³ except that a phenol-chloroform (1:1) mixture was used for extracting the proteins. Nuclease *S*₁ was prepared from *Aspergillus oryzae* crude α -amylase (Sigma) by the method of Sutton⁴. The reaction mixture consisted of 30 μ g of native or heat-denatured DNA, and 10 μ g nuclease *S*₁ all in 1.5 ml of KZS (0.1 M KCl, 0.1 mM ZnSO₄, 0.025 M sodium acetate pH 4.5). Incubation was at 37°C for 30 min. After chilling, 25 μ g of carrier calf thymus DNA was added followed by 0.5 ml of 2 N perchloric acid. The control system (1.5 ml) consisted of 30 μ g of liver DNA, 10 μ g enzyme in KZS to which 25 μ g carrier DNA and 0.5 ml of 2 N chilled perchloric acid were added without incubating. The acid-insoluble material was collected by centrifuging at 17,000 r.p.m. for 20 min. The *A*₂₆₀ of the supernate was measured. The amount of DNA rendered acid-soluble by nuclease *S*₁ was obtained by subtracting the *A*₂₆₀ of the control supernate from that of the corresponding experimental sample. This value represented the amount of DNA digested by nuclease *S*₁ and was used to calculate the percentage of hydrolysed DNA. ●, Native DNA; ○, denatured DNA.

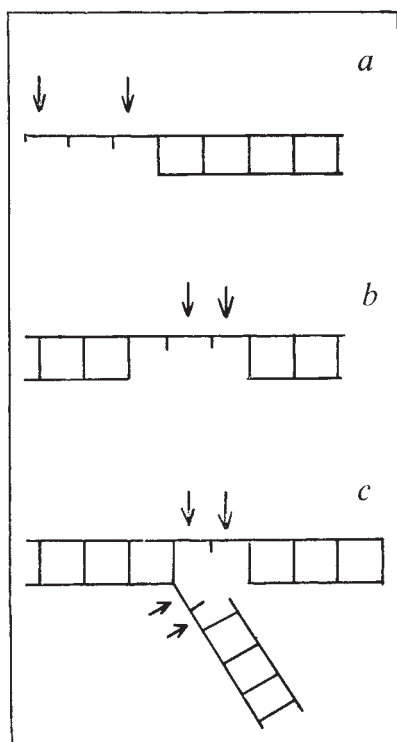


Fig. 2 Postulated model of the regions of nuclease S_1 attack on the DNA of senescent mice. DNA with single-stranded region (a) terminally located, and (b) internally located; (c) cross-linked DNA with single-stranded regions in the junction region. Arrows indicate points of possible nuclease attack.

the formation of regional single-stranded stretches in the DNA of senescent animals represents a transitional stage in the process leading to the elimination of nucleotide sequences that are to be discarded¹. Figure 2 shows models of the possible structures of the regions in DNA attacked by nuclease S_1 . There may be many areas of nuclease S_1 attack on crosslinked DNA molecules of which we present a simpler model in Fig. 2c. The metabolic significance of the existence of single-stranded regions in the DNA of older mice remains unknown. These regions may be the result of increased nuclease activity coinciding in time with a senescence-associated decline in repair enzyme activity in the cells. Loss in enzyme activity during senescence has been observed in other systems⁸. Thermodynamically, this has the advantage of making the energy previously reserved for repair functions available for hydrolytic processes. If the defective regions of the DNA occur in the genes for constitutive functions and are not repaired at the appropriate time, the biochemical lesions in the metabolic pathways of the particular cells are likely to result in the death of the organism; the harm can be particularly severe in the cells of non-replenishing tissues such as those of the muscular and nervous systems.

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Is mRNA transcribed from the strand complementary to it in a DNA duplex?

WE have tested the assumption that the messenger RNA sequence is uniquely determined by the DNA sequence complementary to it. Because RNA is usually transcribed from a DNA duplex, there is the possibility that the DNA strand that is not complementary to the mRNA influences the RNA base selection. This possibility exists in models that postulate like-with-like base pairs¹⁻³, triple helix models of DNA-DNA-RNA⁴, triple base interactions such as those described in tRNA⁵, and models in which RNA polymerase may confer a special type of specificity (non-Watson-Crick) to RNA base-DNA duplex interactions, as well as in experiments in which separated are compared with non-separated strands⁶⁻⁷.

The logic of our experiment is as follows. If one makes DNA heteroduplexes in which one strand has the coding for the premature termination of a given protein, while the other has the coding for the complete protein, and then uses these heteroduplexes as templates for transcription-translation in a coupled⁸ cell-free protein synthesizing system, then one can ask, by fractionating the radioactive protein products on Ornstein-Davis⁹⁻¹⁰ acrylamide gels containing SDS, which product is made—the complete protein, a fragment or neither? If coding for RNA synthesis were solely a function of the nucleotide sequence of only one strand of a DNA duplex, then no matter

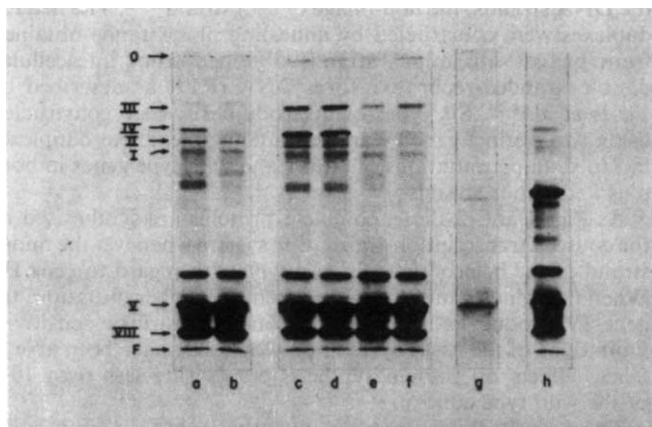


Fig. 1 Autoradiograph of urea SDS-Tris-glycine acrylamide gels showing the *in vitro* protein products stimulated by various homo- and hetero-duplex DNAs. Gene products were identified as described before¹⁸ and are marked with Roman numerals. DNAs used as template for incorporations shown in (a), (b) and (h) were homoduplex (had not been subjected to cleavage, denaturation and renaturation). DNAs used were: (a) WT homoduplex; (b) amber IV₁₂ and VII homoduplex; (c) amber IV₁₂ and VIII +, WT -; (d) amber IV₁₇ +, WT -; (e) WT +, amber IV₁₂ and VII -; (f) WT +, amber IV₁₇ -; (g) background (no added DNA: the bands reflect the synthesis of *E. coli* proteins from endogenous message); (h) amber IV₁₇ homoduplex, showing prominent fragment produced when this DNA is used as template. DNA was isolated as described before¹⁸. Heteroduplex molecules were constructed by cleaving RFI with endonuclease R-Hind, isolating the cleavage products on sucrose gradients, denaturing the product in alkali, annealing to a 20-fold molar excess of the appropriate phage (+) strand DNA, and re-annealing at 65°C for 1.5 h, as described by Vovis *et al.*¹³⁻¹⁴. The circular reannealed molecules were purified by neutral sucrose gradient centrifugation and used as templates in the coupled transcription-translation system. All subsequent steps were as described previously¹⁸.

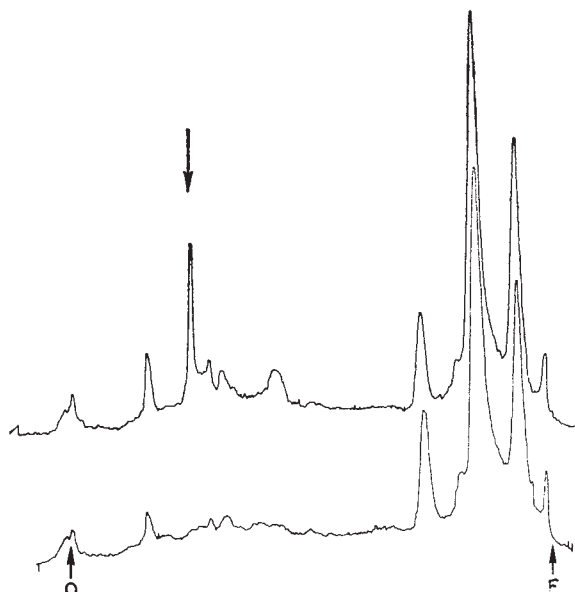


Fig. 2 Densitometer tracing of an autoradiograph of protein products programmed by heteroduplex DNA of composition: +, wild type, -, amber IV (lower tracing); +, amber IV, -, wild type (upper tracing). Other details are as described in Fig. 1. The arrow marks the position of the gene IV product.

which or how many termination signals were on the other strand a complete protein would be made from at least one of the two possible heteroduplexes. Furthermore, if the results indicate that only one strand actively codes for RNA and the resultant protein, one can determine which strand is active.

To test these hypotheses, we constructed heteroduplex DNA molecules using coliphage ϕ 1 DNA. RNA transcribed from this DNA, both *in vivo* and *in vitro*, is complementary to only one of the DNA strands, the non-phage or (-) strand¹¹⁻¹². The heteroduplexes were constructed by annealing plus strands obtained from phage with minus strands obtained from intracellular double-stranded-replicative form DNA (RFI) as described by Vovis *et al.*¹³⁻¹⁴. Six types of heteroduplexes were constructed using two distinct gene IV amber mutants; these heteroduplexes included all permutations of amber and wild type genes in both plus and minus strands.

As Figs 1 and 2 show, complete proteins are synthesised by the coupled transcription-translation system whenever the minus strand of the heteroduplex is wild type with regard to gene IV. When the minus strand contains a gene IV amber mutation, the gene IV product is not made. Because of intrinsic sensitivity limitations of the assay system, we would not have been able to detect effects on protein synthesis comprising less than 10% of the wild type control.

These results thus support the hypothesis that the information for RNA transcription of structural genes is not strongly affected by a single base change in the duplex strand not complementary to the mRNA synthesised. We do not know what the effect of other sorts of changes would be.

All of the heteroduplex molecules were constructed by a method which includes the use of endonuclease R·Hind to cleave ϕ 1 RFI. This endonuclease makes a single, unique double strand break in this DNA¹⁵, which is located within gene II¹⁶⁻¹⁷. Such cleaved DNA does not act as template for the synthesis of gene II protein *in vitro*¹⁸. It is interesting then, that some gene II product was synthesised in reactions primed by the circular heteroduplex DNA molecules which initially contained a single-stranded break within gene II. This suggests that transcription may not be affected by the presence of a nick in the template strand, although further experiments are needed to establish the point firmly.

This experiment was designed to test the transcription rules for structural genes coding for proteins, and showed that only the template strand seems to be significant. DNA which is

heteroduplex in regions corresponding to promoters, operators or transcription termination regions might not function normally, since in these instances DNA-protein recognition is quite likely to involve both strands of a duplex, and there is no *a priori* reason to think that function is limited to one strand.

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Template activity of ϕ 1 RFI cleaved with endonucleases R·Hind, R·EcoP1 or R·EcoB

ENDONUCLEASE R·Hind makes a single, unique, double-stranded break in the double-stranded circular DNA (RFI) which can be isolated from bacteria infected with bacteriophage M13 (ref. 1), ϕ d (refs 2 and 3), or ϕ 1 (Horiuchi and Zinder, unpublished observations). We wish to report that ϕ 1 RFI cleaved by endo R·Hind acts as a template for protein synthesis in a coupled *in vitro* transcription-translation system⁴, and that with the exception of the product of gene II of ϕ 1, the normal complement⁵ of *in vitro* phage-specific proteins is made. No prominent new polypeptide is observed (Fig. 1); this suggests that a substantial fragment of the gene II product is not coded for by RFI cleaved with endo R·Hind. In addition, neither the size nor the relative yield of the product of the proximal gene^{5,6}, gene IV, is affected. This implies that the break is likely to be in a region extending from the C-terminus of gene IV to the N-terminal portion of gene II. A similar location has been assigned to this break with the use of entirely different methods by Seeburg and Schaller⁷.

Endo R·Hind is one of a class of endonucleases which cleave DNA at specific sites and do not require a specific cofactor^{8,9}. The full length, linear, double-stranded DNA

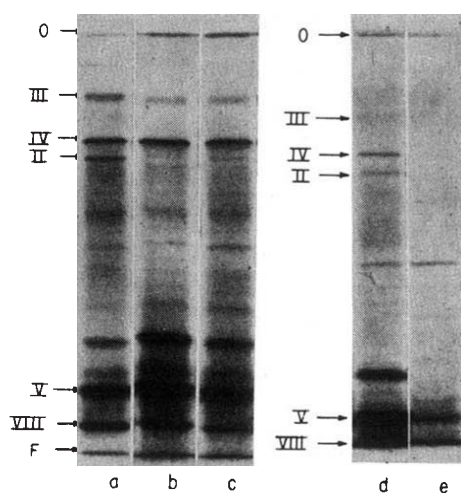


Fig. 1 Autoradiographs of urea-Tris-glycine-SDS acrylamide gels showing the protein products synthesised *in vitro* in a coupled system⁴ with the use of f1 RFI (a), f1 RFI cleaved with endonuclease R·Hind (b), or with endonuclease R·EcoP1 (c), or with endonuclease R·EcoB (d). A background incorporation (no added DNA) is shown in (e), and is to be compared with (d) only. Roman numerals identify gene products, as in ref. 5. (a), (b) and (c) are from the same gel slab, (d) and (e) from a different gel slab. In (d) the coat protein, product of gene VIII, has migrated close to or at the front, and is not readily distinguishable from it. RFI was prepared as before⁵, subjected to cleavage as detailed in the legend to Fig. 2, and used as a template as described before⁵. Electrophoresis, autoradiography and so on were as described before⁵. O marks the origin, F the front.

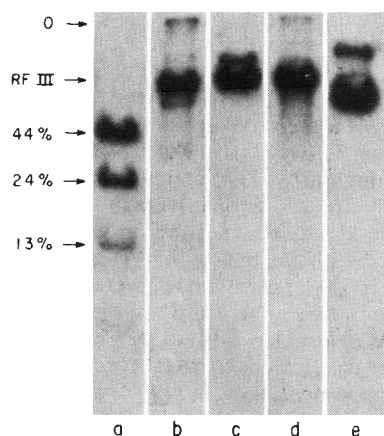


Fig. 2 Autoradiograph of a 1.4% agarose gel showing: a, Three fragments generated from f1 RFI by endonuclease R·HaeIII, as DNA size markers. The size of each fragment is calculated from its relative content of ³²P, and is expressed as a percentage of the total f1 genome. Fragment sizes from Horiuchi, unpublished. b, Endo R·Hind cleavage product of f1 RFI previously cleaved with endo R·EcoP1 (RFIII/P1). c, f1 RFI cleaved with endo R·EcoP1 only. d, f1 RFI cleaved with endo R·Hind only. e, Untreated f1 RFI (the upper band is contaminating RFII). Cleavage reactions with *Haemophilus* endonucleases were carried out as follows: a 20 µl reaction contained 7 mM Tris-HCl, pH 7.4, and 7 mM MgCl₂, 0.2 µg ³²P-f1 RF and either 0.6 µg endo R·HaeIII or 1.3 µg endo R·Hind. Incubations were at 37° C for 1 h. Reactions were stopped by adding 50 mM EDTA-0.25% SDS and the mixture was analysed by electrophoresis on 1.4% agarose gels¹². Cleavage reactions with endo R·EcoB and R·EcoP1 were carried out as described before¹⁰ and the RFIII generated in these reactions was isolated and purified as described¹⁰. The RFIII was dialysed against 0.05 M Tris, 0.05 M NaCl, 0.001 M EDTA, pH 7.4, concentrated under a stream of N₂ gas, precipitated with 3 volumes of 95% ethanol, redissolved in the above buffer and used either as template for protein synthesis or as substrate in further restriction enzyme reactions.

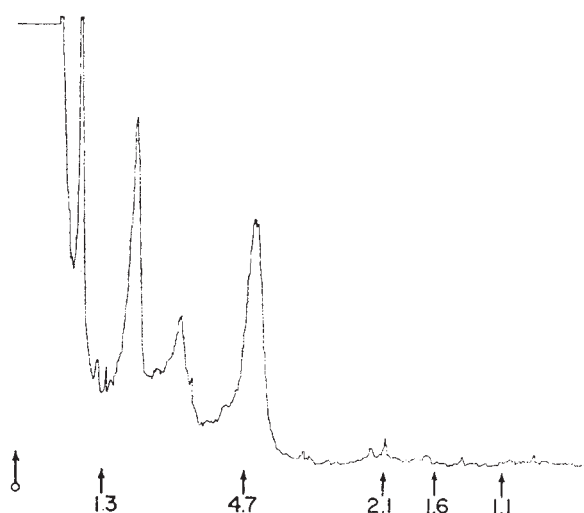


Fig. 3 Densitometer tracing of an autoradiograph of a 4% acrylamide gel slab on which the products generated from ³²P f1 RFI by the action of endo R·EcoP1 and R·Hind had been subjected to electrophoresis. The origin is marked O, the direction of electrophoresis is from left to right. Larger fragments stay at or near the origin, smaller ones migrate into the gel. The arrows indicate the position at which fragments generated by endo R·HaeIII migrated in another slot of the same gel. The numbers under the arrows refer to the sizes of these marker fragments, expressed as a percentage of the f1 genome. All other conditions were the same as those in Fig. 2, and the material placed on the gel was the same as that shown in Fig. 2b. Gels were prepared and run as described by Danna *et al.*¹³.

(RFIII) produced from f1 RFI by the action of endo R·Hind, when denatured and subsequently reannealed, remains a linear, full length molecule¹. We have previously shown¹⁰ that in the presence of S-adenosyl methionine and ATP, endo R·EcoB and R·EcoP1 also yield full length linear molecules as their primary product, but, in contrast to those produced by endo R·Hind these molecules form circular structures upon denaturation and renaturation. This observation suggested that the sites of cleavage of these latter enzymes are not unique, even though only one double-strand break is introduced into any particular molecule¹⁰.

It was interesting, then, to use f1 RFIII produced by these enzymes as templates for the coupled system. As Fig. 1 shows, endo R·EcoP1, like endo R·Hind creates an RFIII which is no longer able to act as template for the synthesis of gene II product. DNA cleaved by endo R·EcoB seems still to code for the normal complement of f1 *in vitro* proteins, but at much reduced efficiency. In view of the postulated heterogeneity of the cleavage sites for endo R·EcoP1 (ref. 10), the specific absence of gene II protein was rather surprising. Our dilemma was resolved, however, by examination of the cleavage products obtained when RFI is treated both with endo R·EcoP1 and R·Hind. Figure 2 shows that stepwise treatment of f1 RFI with endo R·EcoP1 and then endo R·Hind generates a primary product which is not distinguishable, in mobility on 1.4% agarose gels, from that produced by endo R·Hind alone. Longer exposure of the autoradiographs shows that in addition, two minor components are found as products of the combined cleavage, one comprising some 70% and the other about 30% of the genome. When the products of the stepwise cleavage are analysed on 4% acrylamide gels, which resolve smaller fragments, four additional fragments are observed which range in size between 4 and 16% of the f1 genome (Fig. 3). We interpret these results to mean that most of the endo R·EcoP1 mediated breaks occur at positions which are within a distance of 4 to 16% from the site of the endo R·Hind break. The other cleavage products of the combined action of the two enzymes, those ranging in size from 84 to 96% would not be readily

distinguishable from the full length RFIII in the agarose gels (Fig. 2). From the protein synthesis data, we infer that most of the endo R-*Eco*PI breaks occur within gene II, which spans, at a minimum, some 20% of the f1 genome⁵. The site which gives rise to the 70 and 30% fragments is most probably not within gene II.

Takanami¹¹ has demonstrated that *in vitro* transcription of fd RFI leads to at least four well defined transcripts. Use of fd RFI cleaved by endo R. *Hind* diminished the size of the two larger transcripts, but did not affect the size of the two smaller². These results of Takanami², which we have confirmed, together with the data presented here, suggest that only the larger of the RNAs participate in the synthesis of gene II protein.

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Induction of dominant lethal mutation in male mice by ethyl alcohol

THERE have been many studies of the effects of ethyl alcohol on reproductive performance and of the genetic aspects of this relationship¹⁻¹⁰. But in spite of extensive investigations of the genetic determination of alcohol preference in experimental animals, there has been little research on other genetic aspects of alcohol consumption¹¹⁻¹³. It has been assumed for a long time that alcohol presents no genetic hazard. Using the dominant lethal technique, we have now found that in mice ethyl alcohol can be mutagenic when given under certain conditions.

We carried out three experiments to investigate the mutagenic role of ethyl alcohol in male mice. In the first (done in the Department of Genetics, University of Cambridge, England) eight 10-week-old CBA/Fa Cam male mice were given 0.1 ml of 40% ethyl alcohol (1.24 g g⁻¹) by polyethylene gastric tube once a day for 3 consecutive days.

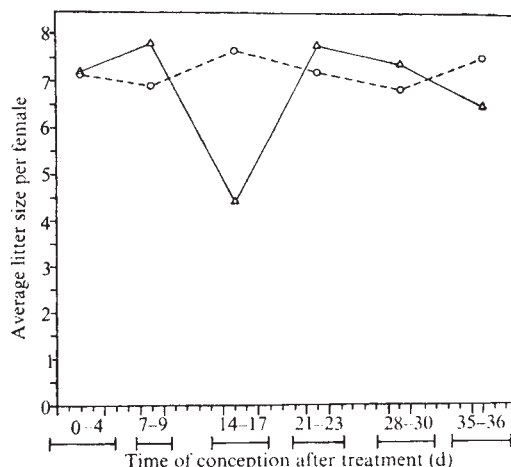


Fig. 1 Effect of ethyl alcohol on litter size sired by treated males. ○, control; △, treated.

Each mouse was then caged with a virgin, untreated female of the same strain. On the seventh day the males were transferred to cages with different virgins. Five treated males were allowed to mate with new females at weekly intervals until the sixth week. Pregnant females were allowed to produce their first litters, the size and sex ratios of which were recorded. The young were examined for morphological abnormalities. Several matings of the breeding colony were set up at the same time and in the same way but without alcohol treatment, to serve as a control.

In our second experiment (carried out at the Worcester Foundation for Experimental Biology) mice of the inbred strain CBA/J (Jackson Laboratory) were tested for mutagenicity of ethyl alcohol using the dominant lethal test. Mice were raised at the Worcester Foundation from the age of 7-10 weeks before use. Two groups of male mice were given ethyl alcohol by gastric tube as before. In one group, 13 males were treated with 0.1 ml of 40% alcohol daily for 3 consecutive days. The second group of six males were each treated as above but with 0.1 ml of 60% (1.88 g g⁻¹) alcohol. The eight control males were each given 0.1 ml of distilled water. After the third treatment, each was mated to two CBA/J females and to further females after 3 d. Each male was allowed to mate with new females seven more times at 4-d intervals. The results of such a mating schedule would reflect the differential response of the different maturation stages of the germ cells.

Successful matings were established by observing the formation of the vaginal plugs. Pregnant females were killed and dissected 13-15 d after conception. Corpora lutea and

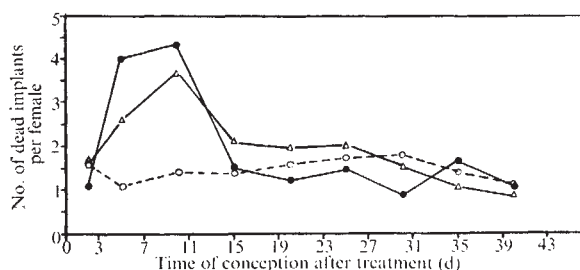


Fig. 2 Dominant lethal induction in male mice with ethyl alcohol. Effect on dead implants. ○, control; △, treated with 40% alcohol; ●, treated with 60% alcohol.

Table 1 Size of litters sired by male mice treated with 0.1 ml of 40% ethyl alcohol

Group of animals	Time of mating after treatment (d)	No. of females mated	Frequency of fertile mating (%)	Litter size (mean $\pm$ s.e.)	Dominant lethal* mutation index
Control	0-42	60	95	7.2 $\pm$ 0.42	—
Treated with alcohol	0-4	10	100	7.8 $\pm$ 0.36	-8.3
	(1st week)				
	7-9	10	100	7.5 $\pm$ 0.50	-4.2
	(2nd week)				
	14-17	10	100	4.4 $\pm$ 0.80†	38.3
	(3rd week)				
	21-23	10	90	7.7 $\pm$ 0.33	-6.5
	(4th week)				
	28-30	10	100	7.3 $\pm$ 0.92	-1.4
	(5th week)				
	35-36	10	100	6.4 $\pm$ 0.48	11.1
	(6th week)				

*Dominant lethal mutation index

$$= \left[1 - \frac{\text{Litter size per experimental female}}{\text{Litter size per control female}} \right] \times 100$$

†t test for comparison between experimental and control values ($t=3.145$, $P<0.01$).

dead and live implants were scored for each pregnancy. Dead implants refer to early deaths. Late embryonic deaths were included with the live implants. The frequency of induced dominant lethal mutations was calculated according to Ehling *et al.*¹⁵.

To assess the reproducibility of our results we repeated the second experiment, testing the effect of only one dose of alcohol, using CBA/J mice. Twelve males were treated with 40% alcohol. Twelve control mice received water instead of alcohol.

The size of full term litters obtained in the first experiment gives an estimate of the induction of dominant lethals as shown in Table 1. The number of live born approximates closely the number of live implants observed at mid-pregnancy except for late deaths. The latter has been found to be similar in mice treated with mutagenic agents and their control¹⁶⁻¹⁸.

The frequency of fertile matings in both control and treated groups seems not to be affected, particularly in the latter. Treatment of female mice of the same strain with the same dose of alcohol but for a longer period did not affect their fecundity (F.B., R.S.B. and M. E. Wallace, unpublished results). Prolonged treatments with larger doses were, however, reported to have some effect¹¹.

There is a close similarity between the average litter sizes of the control and the treated groups, with the exception of litters produced from matings that took place 14-17 d after treatment (Fig. 1). The difference in litter size of that group and the control is significantly different ($t=3.15$; $P<0.01$). The estimated dominant lethal mutation index is 38.3. The results suggest a considerable loss during prenatal life which could be, in large measure, the result of the induction of dominant lethals by alcohol. The results also suggest that different stages of germ cell maturation respond differently.

Although the matings were established at specific times, the entire period necessary to discern the precise pattern of differential response through spermatogenesis was not covered. A similar effect of alcohol on prenatal mortality has been reported with guinea pigs¹⁴, mice⁹ and rats¹².

A male that mated 10 d after cessation of treatment sired one young with abnormal bulgings on the head. The mouse was underweight for its age and died a few days after birth. All of its sibs were normal. The mother of the deformed mouse mated with the same treated father produced three more litters, none revealing similar or other types of abnormalities. It would be premature to conclude that this abnormality was due to alcohol treatment of the father.

Various abnormalities have been reported among progeny of animals treated with alcohol but in no case was there evidence that the alcohol caused any genetic changes^{7,13,19}.

Our second and third experiments were designed to obtain more specific information on the role of ethyl alcohol as a compound potentially able to induce dominant lethal mutation in the mouse. The two sets of data obtained from each experiment were analysed separately. The homogeneity of the data derived from the two experiments was

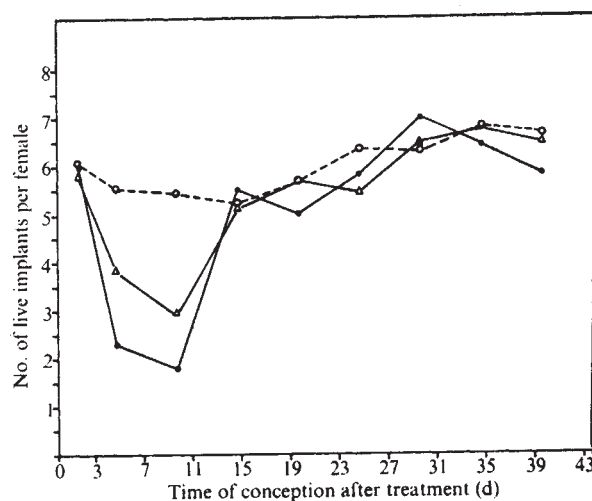


Fig. 3 Dominant lethal induction in male mice with ethyl alcohol. Effect on live implants. Symbols as in Fig. 2.

also tested and no statistical differences were found. This justified pooling the results of these two experiments presented in Table 2. The number of eggs shed, as represented by the number of corpora lutea at mid-term pregnancy, was not significantly different in the control and treated groups. Similarly, there was no significant difference in the number of total implants between control and treated groups.

The most striking observation is the significant (two to fourfold) increase in the number of dead implants in females mated 4-13 d after treatment of males with alcohol.

Table 2 Induction of dominant lethal mutation by ethyl alcohol in male mice

Treatment	Time of mating after treatment (d)	No. of females with implants	Corpora lutea per female (mean $\pm$ s.e.)	Total implants per female (mean $\pm$ s.e.)	Dead implants per female (mean $\pm$ s.e.)	Live implants per female (mean $\pm$ s.e.)	Dominant* lethal mutation index
Control							
Water	1-43	165	9.0 $\pm$ 0.2	7.5 $\pm$ 0.3	1.5 $\pm$ 0.2	6.0 $\pm$ 0.3	
40% Ethanol	1-3	36	8.7 $\pm$ 0.3	7.5 $\pm$ 0.4	1.7 $\pm$ 0.2	5.8 $\pm$ 0.4	4.9
	4-8	33	9.5 $\pm$ 0.2	6.5 $\pm$ 0.6	2.6 $\pm$ 0.2†	3.9 $\pm$ 0.6†	30.6
	9-13	36	9.2 $\pm$ 0.3	6.6 $\pm$ 0.6	3.7 $\pm$ 0.3†	2.9 $\pm$ 0.6†	46.3
	14-18	38	9.1 $\pm$ 0.3	7.2 $\pm$ 0.5	2.1 $\pm$ 0.3	5.1 $\pm$ 0.6	1.9
	19-23	32	8.7 $\pm$ 0.3	7.7 $\pm$ 0.4	2.0 $\pm$ 0.3	5.7 $\pm$ 0.6	0.0
	24-28	26	9.4 $\pm$ 0.2	7.4 $\pm$ 0.6	2.0 $\pm$ 0.3	5.4 $\pm$ 0.4	15.0
	29-33	23	9.8 $\pm$ 0.3	8.0 $\pm$ 0.4	1.5 $\pm$ 0.4	6.5 $\pm$ 0.5	-3.2
	34-38	22	9.5 $\pm$ 0.4	7.7 $\pm$ 0.5	1.0 $\pm$ 0.2	6.7 $\pm$ 0.5	1.5
	39-43	24	9.5 $\pm$ 0.4	7.3 $\pm$ 0.7	0.8 $\pm$ 0.2	6.5 $\pm$ 0.5	3.0
60% Ethanol	1-3	13	9.0 $\pm$ 0.4	7.1 $\pm$ 0.7	1.1 $\pm$ 0.4	6.0 $\pm$ 0.7	7.7
	4-8	8	9.3 $\pm$ 0.6	6.3 $\pm$ 0.4	4.0 $\pm$ 0.8*†	2.3 $\pm$ 0.8*†	57.4
	9-13	11	8.9 $\pm$ 0.4	6.1 $\pm$ 0.7	4.3 $\pm$ 0.4*†	1.8 $\pm$ 0.6*†	67.3
	14-18	8	9.5 $\pm$ 0.5	7.0 $\pm$ 0.8	1.5 $\pm$ 0.3	5.5 $\pm$ 0.5	-14.6
	19-23	12	9.0 $\pm$ 0.4	6.2 $\pm$ 0.5	1.2 $\pm$ 0.1	5.0 $\pm$ 0.8	-2.0
	24-28	7	8.4 $\pm$ 0.7	7.2 $\pm$ 0.6	1.4 $\pm$ 0.2	5.8 $\pm$ 0.6	4.9
	29-33	8	8.7 $\pm$ 0.7	7.8 $\pm$ 0.6	0.8 $\pm$ 0.1	7.0 $\pm$ 0.8	-11.1
	34-38	11	8.6 $\pm$ 0.3	8.0 $\pm$ 0.7	1.6 $\pm$ 0.2	6.4 $\pm$ 0.7	4.5
	39-43	9	8.0 $\pm$ 0.3	6.8 $\pm$ 0.7	1.0 $\pm$ 0.3	5.8 $\pm$ 0.3	0.0

*Dominant lethal mutation index

$$= \left[1 - \frac{\text{Live implants of experimental group per female}}{\text{Live implants of control group per female}} \right] \times 100$$

†Statistically significant from the control values, $P < 0.01$.

This considerable increase in post-implantation death is associated with a proportional decrease in the number of live embryos. These differences between the treated and control groups were statistically significant.

The dominant lethal mutation index was calculated using the formula given in Table 2. The values were based on comparison of the experimental groups of a specific mating interval and their concurrent control group. The estimated dominant lethal mutation index increased for matings between days 1 and 13 in the 40% group, reaching a peak during days 9 to 13. For groups treated with 60% alcohol, the increase in dominant lethal mutation index was rapid and abrupt with much higher frequencies at the mating intervals of 4-8 and 9-13 d. These frequencies (57.4 and 67.3) are higher than for groups treated with 40% alcohol (30.6 and 46.3) at the same mating intervals, which suggest in turn a positive dose response relationship. These results show that ethyl alcohol in the doses used induced dominant lethal mutations in several different spermatogenic stages, namely epididymal spermatozoa and late spermatids. The efficiency of induction is, however, more pronounced in the late spermatid stage.

Although the results derived from both experiments, the one based on litter size and the other on uterine contents, point to differences in the most sensitive stages of spermatogenesis both tests reveal the mutagenic nature of ethyl alcohol.

Dominant lethal mutations have been regarded as imposing no genetic hazards on man since they merely result in abortions. Whether ethyl alcohol could produce a wider range of genetic effects beside dominant lethals, needs further investigation. A pilot study on heavy drinking males indicates a significantly higher correlation between the incidence of birth defects and the drinking behaviour of the fathers (F.M.B., S. Beaton and R. Trudell, unpublished). Association between maternal alcoholism and aberrant morphogenesis in the offspring has also been described recently²⁴.

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Evidence for postmeiotic effect of *t* factors causing segregation distortion in mouse

THE *t* complex of the mouse consists of a large series of genetic factors often characterised by embryonic lethality of the homozygotes, reduced male fertility of viable homozygotes, suppression of recombination over a relatively long chromosomal segment and relatively frequent occurrence in natural mouse populations. Most *t* factors also possess a component which interacts with the *Brachyury* (*T*) gene and causes complete absence of the tail in *T/t* heterozygotes. One of the most fascinating but least explored characteristics of the *t* factors is their effect on genetic segregation. A male mouse carrying a

Although this segregation distortion has been known for almost half a century, it has not been satisfactorily explained. It has been suggested that the distortion may be due to abnormal meiotic divisions (meiotic drive¹). More specifically, it may be due to a process in which homologous chromosomes distribute themselves unequally during metaphase I of male meiosis with the result that the spermatocytes receive one chromosome of a given pair more frequently than the other. Here we present evidence that in a *t/+* male, the *t* and the *+* bearing chromosomes are distributed during metaphase I in a 1:1 ratio, implying that the actual distortion must occur after metaphase I, most likely postmeiotically.

The evidence for the meiotic effect of *t* factors was obtained by cytogenetical means, using a strain of mice recently produced in this laboratory and carrying Robertsonian translocation *T(16;17)7Bn*, abbreviated *T7* (ref. 2). The strain was derived by repeated backcrossing of hybrids between the house mouse (*Mus musculus*) and the tobacco mouse (*M. poschiavinus*) to the inbred strain C57BL/10, and selection for chromosome 17 carrying the *t* complex. During the selection, six of the seven metacentrics present in the tobacco mouse were eliminated and only the one composed in part of chromosome 17 was maintained. The *T7* translocation is easily identifiable both at mitosis (as a single or double metacentric among 39 or 38 telocentrics, depending on the zygosity of the carrier) and meiosis (as a quadrivalent at metaphase I and a metacentric chromosome at metaphase II).

The rationale for the present experiment was as follows: if the segregation distortion is caused by meiotic drive during metaphase I, at metaphase II a *T7+/-t* male should show a preponderance of meiotic figures lacking the metacentric marker; if, on the other hand, the distribution of homologous chromosomes at metaphase I is normal, a 1:1 ratio of figures with and without the metacentric can be expected at metaphase II.

The prerequisite of such an experiment is that the *T7* translocation itself does not greatly disturb the segregation of the homologues at meiosis. For this reason, males carrying the *T7* marker but not the *t* factor were also tested.

Table 1 Frequency of metaphase II spreads with one metacentric chromosome in *T7+/-t* males

Animal Number	1M*	OM†	Total	%1M‡	χ ²
199/919	9	12	21	42.9	0.42
199/920	32	37	69	46.4	0.32
199/921	11	17	28	39.3	1.28
199/922	28	36	64	43.8	1.0
199/923	27	32	59	45.8	0.42
199/924	21	27	48	43.8	0.76
199/925	19	30	49	38.8	2.46
200/1151	7	11	18	38.9	0.88
200/1334	29	41	70	41.4	2.06
Total	183	243	426	43.0	8.4

* No. metaphase II spreads with one metacentric (*T7*).

† No. metaphase II spreads without the metacentric.

‡ % spreads with one metacentric.

t factor in a heterozygous condition (*t/-*) often transmits the factor to almost 100% of its progeny, in defiance of the Mendelian principle requiring 1:1 segregation.

Table 2 Comparison of results with different *t* factors

Genotype	No. of males tested	Frequency of metaphase II spreads with one metacentric chromosome in males of indicated genotype				Segregation of the metacentric chromosome to offspring of males tested for the presence of this chromosome in metaphase II				Segregation of <i>t</i> factor in the absence of the metacentric chromosome	
		1M/T*	%1M†	χ ² ‡	χ ² §	1M/T	% progeny with 1M	χ ² ¶	χ ² **	Ot/T††	%Ot‡‡
<i>+ + / + +</i>	1	4/69§§	5.8	54.0	46.4						
<i>T7 + / + +</i>	1	57/122	46.7	0.52							
<i>T7 + / + T</i>	5	70/155	45.2	1.46	0.15	73/137	53.3	0.60			
<i>T7 + / + t^{w2}</i>	1	21/47	44.7	0.54	0.08	1/18	5.6	14.2	0.47	15/137	10.5
<i>T7 + / + t^{w3}</i>	5	85/116	42.3	4.8	1.57	2/67	3.0	59.2	3.03	4/44	9.1
<i>T7 + / + t⁶</i>	9	183/426	43.0	8.4	2.38	10/177	5.6	139.2	65.92	25/72	34.7
<i>T7 + / + t¹²</i>	4	78/170	45.9	1.16	0.04	2/92	2.2	84.2	23.88	49/205	23.9

*Number of metaphase II spreads with one metacentric (*T7*)/total number of scored spreads.

†% spreads with one metacentric.

‡Observed frequencies of 1M spreads were compared to expected frequencies (that is 50%).

§Observed frequencies of 1M spreads were compared to observed frequencies of 1M spreads in *T7+/-+* male.

||Number of progeny with one metacentric/total number of progeny. Progeny arose from matings of mice with the genotype indicated in the first column to *+ + / + +* or *+ + / + T* mice. Presence or absence of the metacentric chromosome (*T7*) was determined in mitotic metaphase spreads.

¶Observed frequency of progeny with *T7* from the matings of *T7+/-T* or *T7+/-t* × *+ + / + +* or *+ + / + T* was compared to expected frequency (that is 50%).

**Observed frequency of progeny with *T7* from the mating of *T7+/-t* × *+ + / + +* or *+ + / + T* was compared to observed frequency of progeny from the mating *+ + / + t* × *+ + / + +* or *+ + / + T* (see column 12).

††Number of progeny without *t*/total number of progeny. Progeny arose from matings of *+ T / + t* or *+ + / + t* to *+ + / + +* mice. Segregation of *t* chromosome was detected by H-2 typing.

‡‡% progeny without *t* from matings in the absence of *T7*.

§§As B10.A lacks the metacentric chromosome, the four spreads scored as 1M must be reading errors. Two telocentric chromosomes which happen to be positioned in the spreads close to each other with their centromeres almost in contact and their arms pointing in opposite directions resemble and are confused with a metacentric chromosome. Such a reading error, however, is so small that it does not influence the conclusions drawn from the data.

In the experiment, $T7+/T7+$ females were mated to $+t/+T$ males and two types of progeny were produced: $T7+/+t$ (normal tail) and $T7+/+T$ (short tail). Both the normal tail and the short males were further mated to $+/++$ or $+/+T$ females; the progeny of these matings were killed, mitotic chromosome preparations were prepared from bone marrow³ and the segregation of the $T7$ chromosome was determined. This progeny test provided the necessary control for demonstrating that the t factor distorted segregation whereas the $T7$ translocation did not. If this were the case, one would expect the progeny of the $T7+/+T \times +/++$ mating to segregate in a 1:1 ratio of mice with and without the metacentric, and the progeny of the $T7+/+t \times +/++$ mating to have preponderance of mice without the metacentric. After a sufficient number of progeny were obtained from the $T7+/+T$ and $T7+/+t$ males, the animals were killed meiotic chromosome preparations were made from their testes⁴, and the metaphase II spreads evaluated for the presence or absence of the metacentric chromosome. Twenty to twenty-five slides were made from each male and only metaphase II spreads of 19 telocentrics and one metacentric, or 20 telocentrics were counted. Four different t factors were tested: t^6 , t^{12} , t^{w2} , and t^{w5} .

Although there was a considerable male-to-male variation in the individual experiments (Table 1), the overall results with the different t factors were comparable (Table 2). In the meiotic test (Table 2), the observed frequency of 1M spreads (that is metaphase II spreads with one metacentric chromosome) in $T7+/++$ mice was not significantly different from the observed frequency of 1M spreads in $T7+/+t$ mice. When the observed frequency of 1M spreads in $T7+/+t$ mice, however, was compared to the expected frequency (50%), significant difference was found for some t factors (t^6 and t^{w5}). The departure from the expected value was most likely caused by the presence of the metacentric chromosome. (The frequency of 1M spreads in $T7+/++$ animals was lower than the expected 50% value, but for the number of animals used, the difference was not statistically significant.)

In the progeny test (Table 2), two t factors (t^{w2} and t^{w5}) were transmitted in almost exactly the same ratios in $T7+/+t \times +/++$ matings as in $+T/+t \times +/++$ or $+/+t \times +/++$ matings. The other two factors tested (t^6 and t^{12}), on the other hand, were transmitted in significantly lower ratios in $T7+/+t \times +/++$ matings than in $+T/+t \times +/++$ or $+/+t \times +/++$ matings. This latter observation suggests that $T7$ interacts with t factors postmeiotically, resulting in an increased transmission ratio of t . This interaction, however, can be detected only with t factors that, in the absence of $T7$, have low (t^6) or moderate (t^{12}) transmission ratios. The nature of the interaction is not known.

We conclude that two types of segregation distortion operate in the experiments we describe; one a very minor type occurring during the first meiotic division and another which has a very strong effect postmeiotically. The meiotic distortion can most likely be attributed to the presence of the $T7$ translocation, whereas the strong distortion is caused by the t factors and it may be enhanced by the $T7$ chromosome. Thus, the distortion resulting from t factors does not occur in metaphase I as a result of a structurally abnormal chromosome but rather postmeiotically. This conclusion is in agreement with the data of the effect of late mating^{5,7}, which shows a normalisation of the transmission ratios as a result of the shorter period of time between insemination and fertilisation. Further evidence for the postmeiotic effect of t factors is provided by recent work⁸ in which epididymal sperm was incubated *in vitro* and the number of surviving sperm were estimated at intervals by a cytotoxic test using antisera directed against a specific t factor. They demonstrated that the number of t bearing sperm increases with time as the number of $+$ sperm decreases.

The data we present, however, do not rule out the possibility of a biochemical activity related to the t factors that may occur before or early in meiosis but have a postmeiotic effect as

has been suggested by Erickson⁷.

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Factor which affects the mode of genetic recombination in *E. coli*

The normal recombination process in *Escherichia coli* requires a pathway involving a functional *recB* and *recC* gene product (henceforth designated the RecBC pathway).¹ The *recB* and *recC* genes map as closely linked cistrons near *thy* on the *E. coli* chromosome map (54 min)^{2,3}. ATP-dependent DNase (*recBC* DNase) has been identified as the product encoded by these genes⁴⁻⁶. Another gene essential for recombination proficiency, *recA*⁷, is located between *cysC* and *pheA* (51 min)⁸. Genetic transformation studies⁹⁻¹² showed that the most efficiently transformable *E. coli* strain (genotype: *recB*⁻ *recC*⁻ *sbcB*⁻) lacks the DNase but retains recombination proficiency because of the *sbcB*⁻ allele which acts as an indirect suppressor of the *recB*⁻ *recC*⁻ mutations¹³. This strain uses a recombination pathway (designated as the RecF pathway)¹ which does not involve the ATP-dependent DNase but consists of products of several recombination genes¹³. Restoration of the ATP-dependent DNase to this strain by introduction of the *recB*⁺ *recC*⁺ alleles reduced the transformation frequency by a factor of approximately five⁹. *In vitro* studies demonstrated that this DNase degrades linear DNA molecules extensively, while it has no effect on circular DNA⁴. The adverse effect of the DNase on transformation may therefore be due to destruction of linear donor DNA molecules before they reach the recombination machinery. These results raise the possibility that in normal cells the DNase affects the mode of recombination by selecting or destroying DNA molecules of a particular structure.

The transfer of genetic material can result in the formation of two possible types of recombinant clones: (a) substitution type resulting from replacement of recipient genetic material by a homologous segment of donor material, and (b) addition type

resulting from addition of the donor genetic material to the recipient chromosome. We describe here transduction and transformation experiments designed to examine the effect of the *recBC*-specified DNase on the two possible types of recombination. Similar experiments using λ gal bio were reported recently by Wackernagel and Radding¹⁴.

The genetic backgrounds of the *E. coli* K12 strains used in our study have been described¹⁰. M0639 is a tryptophan-requiring derivative (*trpB9579*)¹⁵ of JC7623 (*recB21 recC22 sbcB15*)¹⁶. Strains M0649 (*recB⁺ recC⁺ sbcB15*) and M0650 (*recB⁺ recC⁺ sbcB⁺*) were constructed from strain M0639 by conjugation with Hfr KL16.

We studied specialised transduction and transformation with ϕ 80pt and λ pt phages and their DNA¹⁷⁻²⁰. The ϕ 80pt and λ pt we used both lack the *int* and *red* genes which are replaced by part or all of the *trp* operon of *E. coli*. Two classes of Trp⁺ recombinants are generally observed in a single transduction (or transformation) experiment. One class of Trp⁺ recombinants consists of those lysogenised by donor phage by a mechanism similar to that of specialised transduction. Oka *et al.*²⁰ demonstrated that the entire ϕ 80pt genome integrates exclusively at *trp* operon of *E. coli* undergoing addition type recombination by the mechanism proposed by Campbell²¹. The other Trp⁺ class

between 10% and 20% (13.5% for ϕ 80pt, 17.0% for λ pt) of Trp⁺ recombinants were ϕ 80pt or λ pt lysogens, suggesting that these are the products of addition type of recombination. The rest (86.5% for ϕ 80pt, 83.0% for λ pt) were not lysogenic and therefore the products of substitution type of recombination. Second, in strain M0639 (recombination pathway RecF, no *recBC*-encoded DNase) the proportion of Trp⁺ recombinants which are ϕ 80pt or λ pt lysogens is drastically reduced to less than 1% of the total Trp⁺ recombinants so that almost all Trp⁺ recombinants result from substitution type of recombination. Third, in strain (M0649) (*recBC*-specified DNase present plus a functional RecF pathway) the proportion of the Trp⁺ recombinants harbouring ϕ 80pt or λ pt was the same level as that of M0650.

These results demonstrate that the presence of *recBC*-encoded DNase in the cells affects the fate of donor DNA and changes the proportion of two types of recombinants. Two possibilities can be considered to explain these results. First, the recombination process of the RecF pathway (*recB⁻ recC⁻ sbcB⁻*) results primarily in the substitution type of recombination, whereas the RecBC pathway (*recB⁺ recC⁺ sbcB⁺*) catalyses substantial amounts of both types (substitution and addition) of recombination. Second, the increase in the proportion of the

Table 1 Transduction of the *trpB* gene by ϕ 80pt and λ pt

Donor	Recipient strain	Genotype	Frequency of Trp ⁺ transductants ($\times 10^{-3}$)	Fraction of Trp ⁺ transductants harbouring the donor phage
ϕ 80pt	M0639	<i>recB⁻ recC⁻ sbcB⁻</i>	1.50	1/149 (0.7%)
	M0649	<i>recB⁺ recC⁺ sbcB⁻</i>	0.37	15/150 (10.0%)
	M0650	<i>recB⁺ recC⁺ sbcB⁺</i>	0.31	21/155 (13.5%)
λ pt60-3	M0639	<i>recB⁻ recC⁻ sbcB⁻</i>	5.40	1/155 (0.6%)
	M0649	<i>recB⁺ recC⁺ sbcB⁻</i>	1.80	17/155 (11.0%)
	M0650	<i>recB⁺ recC⁺ sbcB⁺</i>	0.56	27/155 (17.0%)

ϕ 80pt transduction was performed according to Oka *et al.*²⁰. λ pt transduction was performed as follows: overnight Luria broth cultures of the recipients were suspended in the same volume of 10 mM MgSO₄ and aerated for 15 min. The λ pt60-3 was added at a multiplicity of infection of 0.1 and the incubation was continued at 37°C for 20 min. The transduction mixtures for both ϕ 80pt and λ pt60-3 were appropriately diluted, plated on selection plates for Trp⁺ recombinants and incubated at 37°C for 48 h. The Trp⁺ transductants were purified by two single-colony isolations on Difco Bacto penassay agar plates before testing for phage-specific characteristics. More than 99% of the original Trp⁺ transductants remained Trp⁺ after the purification steps. The presence of the phage genome in the Trp⁺ recombinants was demonstrated by both the ability to produce the phages and immunity to clear-plaque mutants of the phages. For testing phage production the purified colonies were spotted on Penassay plates, irradiated with ultraviolet light and replicated on a plate having an overlay seeded with the indicator strain MRP1. A clear zone of lysis proximal to the spot was taken to indicate phage production. The test for immunity was performed by cross-streaking the Trp⁺ recombinants against ϕ 80c or λ c126. All the Trp⁺ recombinants immune to the clear plaque phages were induced by ultraviolet irradiation to produce phage whereas all sensitive recombinants produced no phage.

represents the substitution type recombinants in which only the portion of donor DNA bearing the *trp⁺* genes is introduced into the recipient genome. Such recombinants should not acquire ϕ 80- or λ -specific characteristics. Therefore testing Trp⁺ recombinants for the absence or presence of the phage-specific characteristics (phage immunity and inducibility) is a convenient method for determining the nature of the recombination process undergone.

We have tested for these characteristics in the Trp⁺ recombinants obtained from three isogenic Rec⁺ (*trpB⁻*) recipient strains. One recipient (M0650; *trpB⁻ recB⁺ recC⁺ sbcB⁺*) possesses recombination pathway RecBC and is dependent on the intact *recBC* gene-encoded DNase. The second recipient (M0639; *trpB⁻ recB⁻ recC⁻ sbcB⁻*) possesses only the RecF recombination pathway which functions without an intact *recBC*-DNase. The third strain (M0649; *trpB⁻ recB⁺ recC⁺ sbcB⁻*) possesses the two recombination pathways¹³. Table 1 presents the results of transduction experiments involving these strains with ϕ 80pt (*trpA⁺B⁺C⁺*) and λ pt60-3 (*trpA⁺B⁺C⁺D⁺E⁺*) and can be summarised as follows. First, in the wild type (M0650) where the DNase (encoded by *recBC*) is functioning,

addition type recombinants observed in the recipient with the *recBC*-encoded DNase (*recB⁺ recC⁺ sbcB⁺*; *recB⁺ recC⁺ sbcB⁻*) may be the result of decreased availability of a particular type of donor DNA molecule due to its degradation by the DNase. (For example, DNA with either total or partial linear structure, which is sensitive to degradation by the DNase⁴, may be the substrate for the substitution type of recombination. Circular DNA which would survive may be the substrate for the addition type of recombination.) We believe the second possibility is the case for the following reasons. First, the reduction of transduction frequency (3-10-fold) was always observed in the recipient cells with the *recBC*-encoded DNase (Table 1). Second, the frequency of spontaneous segregation of Trp⁻ cells from Trp⁺ lysogens due to the loss of the ϕ 80 and λ were equal (1.5% to 2%) in three recipient cells regardless of the recombination pathway(s) they possessed.

A similar experiment with a specialised transformation system was performed with intact ϕ 80pt (*trpA⁺B⁺C⁺*) DNA as donor and with the same recipients as used for the transduction experiment (except for strain M0650 which is non-transformable). Among the total Trp⁺ transformants the proportion which was

Table 2 Transformation with $\phi 80pt$ DNA

Recipient	Genotype	Frequency of Trp ⁺ transformants ($\times 10^{-4}$)	Fraction of Trp ⁺ transformants harbouring $\phi 80pt$
M0639	<i>recB⁻ recC⁻ sbcB⁻</i>	7.1	8/150 (5.3%)
M0649	<i>recB⁺ recC⁺ sbcB⁻</i>	1.4	104/144 (72.3%)

Specialised transformation was performed as described by Oishi and Cosloy²² using intact $\phi 80pt$ (*trpA⁺B⁺C⁺*) DNA as donor at a concentration of $10 \mu g\ ml^{-1}$. Tests for phage-specific characteristics of the Trp⁺ transformants are described in the legend of Table 1.

the result of lysogenisation by the $\phi 80pt$ (*trpA⁺B⁺C⁺*) genome was distinctly higher with recipient strain M0649 than with strain M0639 (Table 2). This result is essentially the same as we obtained with specialised transduction. With both recipients a much larger proportion of $\phi 80pt$ -bearing Trp⁺ recombinants was obtained after transformation than after transduction, apparently due to a reduced frequency of the substitution type of recombination in transformation. This suggests that DNA molecules which enter the cell as a result of injection by phage tend to interact with the cell's constituents differently from DNA molecules which enter from a pool of naked transforming DNA, perhaps because of differences in structure or in the mode of entry. This interpretation is supported by the report²⁴ that for certain phage the presence of *recBC* DNase in recipient cells reduces the efficiency of transfection, which involves introduction of intact purified phage DNA, while it has little effect on plaque-forming efficiency by infection with intact phage particles.

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Host range of murine xenotropic virus: replication in avian cells

AN endogenous C-type virus with an unusual tropism has been isolated from New Zealand Black (NZB) and other mouse strains. Although it has the murine leukaemia virus (MLV) *gs-1* antigen and reverse transcriptase, it cannot be propagated in mouse cells. It is only infectious for cells foreign to the host species and has been termed xenotropic¹⁻³. In the course of host range studies, we have discovered that, in contrast to other mammalian C-type viruses, this murine xenotropic virus can cross class barriers and both infect and replicate efficiently in avian cells. In these studies, the NZB pseudotype murine sarcoma virus (MSV) (Table 1) was used as it provides a good marker (focus formation) for NZB virus infection and replication.

As illustrated in Table 1, the NZB pseudotype virus gave efficient focus formation in a variety of mammalian cells including human, rat, guinea pig, rabbit, cat, cow, American bear, lion and African water mongoose. Infection occurred also in deer, racoon, gazelle, and Rhesus monkey. In the latter two cell lines only virus replication but not focus formation was detected. Infected animal cell lines which did not demonstrate focus formation nor NZB pseudotype virus production were cocultivated with human cells and passed three times. Supernatants were then assayed again for xenotropic murine sarcoma virus. These human cell cultures were also cocultivated with NRK-Harvey cells to determine if any NZB C-type virus was present. In all cases, the absence of progeny virus production in the original cell culture was not reversed by these added measures.

The xenotropic pseudotype sarcoma virus showed neither focus formation nor replication in C/O chicken cells including those established from *chf*-negative embryos. On the other hand, foci and progeny sarcoma virus were detected in duck, pheasant and quail cells. The foci formed in duck cells differed morphologically from those produced by Rous sarcoma virus (RSV). The MSV focus was composed of elongated, piled up cells rather than the round cells principally seen in RSV foci (Fig. 1a and b). The progeny sarcoma virus maintained the host cell tropism of the parental NZB virus as well as the NZB type specific coat as determined by neutralisation testing using antiserum prepared against the NZB C-type virus^{2,3}. Duck cell cultures yielded the highest titres of progeny virus.

As replication of the NZB pseudotype sarcoma virus requires the presence of a non-focus forming 'helper' C-type virus^{4,6,7},

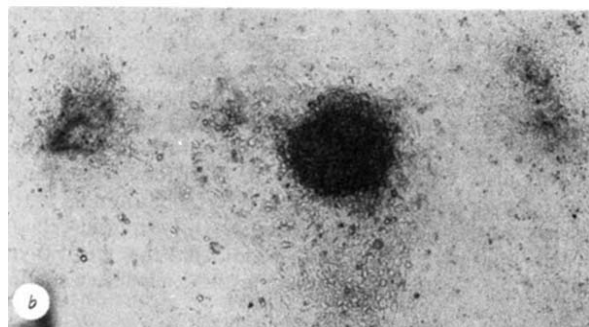
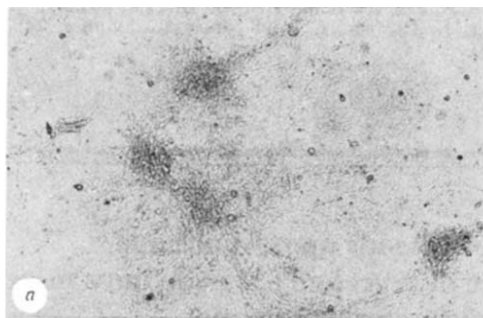


Fig. 1 Focus of cell alteration induced in duck embryo cells, *a*, by the NZB pseudotype murine sarcoma virus (Note the elongated piled up fibroblast-like cells) ($\times 24$); *b*, by the Prague strain of Rous sarcoma virus (note the accumulation of primarily round cells) ($\times 24$).

xenotropic C-type virus must also be produced by these cells. Duck cells were infected directly with the C-type virus isolated from NZB cells and these cultures also yielded progeny NZB xenotropic virus. Duck cell cultures producing xenotropic MSV and/or MLV were superinfected by RSV (type C), and continuous cultures producing both types of viruses have been derived.

Xenotropic viruses isolated from NIH Swiss, C57L and C57BL mice share the same type specific coat as the NZB virus (refs 2 and 3 and P. Arnstein, J. A. L., L. S. Oshiro, P. Price, and E. Lennette, unpublished) and are also able to replicate in duck cells; these data indicate this ability to cross class distinctions is a general characteristic of the murine xenotropic virus(es).

The Harvey strain of murine sarcoma virus as well as Rauscher and AKR pseudotype sarcoma viruses were also tested for their ability to infect duck cells. No foci were detected and supernatants from the cultures did not yield any focus-forming virus nor any MLV as determined by the XC plaque assay⁸. The Kirsten strain of MSV (Ki-MSV) has been reported to have a wide host range including human cells⁹⁻¹², and we have been able to transform duck cells with an isolate obtained from a continuous line of rat cells chronically infected with Ki-MSV. Infection of human cells, however, has not been possible with all preparations of Ki-MSV (J.A.L., unpublished; and S. Panem and W. H. Kirsten, unpublished). Ki-MSV in some laboratories seems to lose its tropism for mouse cells after passage through heterologous hosts (ref. 11 and 12 and V. Klement, personal communication). As suggested previously², it seems most likely, that those preparations of Kirsten MSV contain significant amounts of xenotropic virus and its pseudotype sarcoma virus and these viruses are responsible for the wide host range observed. Neutralisation of the progeny virus from these cultures with antiserum against xenotropic virus is needed to evaluate this possibility. Recently we learned that the isolate we received from Klement has been neutralised by antiserum to xenotropic virus and not antiserum to the parental Kirsten virus (V. Klement, personal communication). This result

suggests that any MLV with a wide host range is most likely from the xenotropic MLV subgroup.

The variability in extent of focus formation and degree of progeny production by the xenotropic pseudotype sarcoma virus indicates that cells differ in their sensitivity for infection by the virus (input response) and their ability to propagate the virus after infection (output response) (Table 1). For example, although the same number of foci can be induced by xenotropic pseudotype sarcoma virus in human and rat cells with approximately the same multiplicity of infection, human cell cultures yield ten times more progeny virus (Table 2) than the rat cell cultures. As MSV replication depends on the helper C-type

Table 1 Host range of xenotropic NZB-MLV

	FF titre*	Progeny production†
Mammalian		
Human foreskin	4.0	3.0
Rat-NRK kidney	3.3	2.0
Guinea pig embryo	3.3	3.0
Rabbit kidney	2.3	3.0
Cat embryo	+	2.0
Bovine embryo	+	3.0
Black footed mongoose	2.9	2.0
African water mongoose	2.0	1.6
Horse dermis	2.3	0.7
American bear lung	2.0	0.3
Lion kidney	1.0	NT
Black footed deer kidney	0.3	0.3
Raccoon uterus	0.2	—
Gazelle lung	—	0.9
Lesser anteater	—	0.5
Rhesus monkey heart (embryo)	—	0.6
Rhesus monkey kidney	—	—
African green monkey kidney	—	—
NIH Swiss mouse embryo	—	—
BALB/c embryo	—	—
Wild mouse kidney (San Francisco)	—	—
Chinese hamster embryo	—	—
Syrian hamster embryo	—	—
Peccary kidney	—	—
Bat lung	—	—
Avian		
Duck embryo (Pekin)	3.0	1.0
Ring-necked pheasant embryo	3.0	0.3
Quail	+	0.3
Chicken embryo (C/O)	—	—

Fertilised duck (Pekin) eggs and lymphomatous-free white Longhorn chicken eggs (C/O) were obtained from Kimberlin Farms (Freemont, California) and used at 10–12 d of age for preparation of primary embryo cultures. Other avian cells were provided by Dr Don Fugita (San Francisco). Cells from the mammalian species were provided by Dr W. Nelson-Rees (Naval Biomedical Laboratories, Oakland). All cultures were maintained and passed according to standard procedures¹⁻⁴.

*The stock of NZB pseudotype sarcoma virus used was made by cocultivating non-virus producing MSV-transformed rat cells, the NRK-Harvey cell line, with NZB embryo cells²⁻³. This virus preparation was adsorbed undiluted for 30 min (37°C) on to the cell monolayers pretreated with diethylaminoethyl dextran⁵. Cell cultures were read for focus formation (FF) on day 7 and 9. The sensitivity of the cell line is expressed by the titre of xenotropic pseudotype virus ($\log_{10}$) obtained. +, Distinct foci could not be detected; —, no foci detected.

†Nine-day supernatants from cell cultures receiving 10^4 focus-forming units of pseudotype virus were assayed for progeny virus on human foreskin cells by standard techniques¹⁻⁴. The titre is expressed as $\log_{10}$. NT, Not tested; —, no progeny virus detected.

virus, these observations suggest that successful infection by xenotropic viruses involves at least two mechanisms; first, at the cell surface before penetration (for example, the receptor level), and second, intracellular after virus penetration. The former depends on the viral envelope coat; the latter on the viral genome. An intracellular block has been proposed to explain the reduced ability of murine N and B tropic viruses to propagate in certain mouse cells¹³. A similar type of block probably limits the replication of xenotropic virus in rat cells,

and other animal cells in which only focus formation is efficient (Table 1). Preliminary results in our laboratory suggest a block at the receptor level prevents xenotropic virus infection of hamster cells, and a block in both mechanisms is responsible for the resistance of mouse cells to the murine xenotropic virus (J.A.L., unpublished).

Table 2 Comparison of progeny yield after infection by NZB pseudotype sarcoma virus

Cells*	Foci†	Progeny yield‡
NRK	150	8
Human foreskin	160	81

*Cells were inoculated at approximately the same multiplicity of infection.

†Numbers represent the average number of foci in duplicate Petri dishes.

‡Seven day supernatants from the cultures were assayed on human foreskin cells. The average number of foci in duplicate plates is given.

The growth of murine xenotropic virus in duck cells represents the first example of a C-type virus from one animal class replicating efficiently in cells from another animal class. Svoboda and Klement reported tumours induced by RSV from which trace amounts of infectious RSV (ref. 14) could be recovered. Recently, the ability of the Schmidt-Ruppin strain of RSV to infect and replicate in kangaroo-rat cells¹⁵ has been described, but the efficiency of infection and replication by this RSV was much less than that of murine xenotropic virus in avian cells. Moreover, after long term culture, the transformed marsupial lines tended to lose their capacity to produce virus¹⁵. On the other hand, duck cells, transformed by the NZB pseudotype virus have increased their virus production with passage and have yielded 10^5 – 10^6 focus-forming particles per ml as measured in either human, rat or duck cells.

The xenotropic MSV can be used as a marker for molecular studies of cells producing both avian and murine C-type viruses. Moreover, the sensitivity of certain avian cells to these murine C-type viruses provides another *in vivo* approach for studying xenotropic viruses and their possible role in normal development, evolution, autoimmunity and/or malignancy.

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Delayed tumour appearance and absence of regression in nude mice infected with murine sarcoma virus

THE murine sarcoma virus (MSV) rapidly induces local tumours in mice^{1,2}. The subsequent regression of such tumours in adult mice has been shown to be immunological in nature^{1,2}. Young animals^{1,2} as well as animals immunosuppressed by X irradiation³, cortisol⁴, cyclophosphamide⁵, antilymphocyte serum⁶ or thymectomy^{2,7} have increased frequency of progressively growing tumours. The immunological nature of these phenomena was also demonstrated by the transfer of resistance to tumour growth with cells⁹ or serum^{2,5,8} from animals that had regressing tumours. The *in vitro* testing of lymphoid cells from mice with regressing tumours has indicated that both thymus-dependent (T)⁹ and non-T lymphocytes¹⁰ are generated as effector cells in this system.

In the work reported here, the capacity to induce tumours and to produce spontaneous regressions of the developing tumours after MSV infection in adult *nu/nu* (nude-athymic) and *nu/+* mice (normal heterozygotes) was studied. Nude mice have an abnormal development of the thymus and thymus-dependent systems and show a profound deficit of immune functions, especially of the thymus-dependent type¹¹.

Nude mice partially inbred in a CBA/H background (5th to 6th backcross generation) and in BALB/c background (8th backcross, 2nd intercross generation) were used, as well as CBA/H or BALB/c mice. (For details of origin of these strains and animal care see ref. 12.) All animals were free of lactic dehydrogenase (LDH)-elevating virus and had normal LDH serum values¹³, and all were injected intramuscularly at 50 d of age with 0.1 ml of Moloney strain MSV (M-MSV). In some experiments the *nu/nu* mice were treated with a thymus graft implanted intraperitoneally at 30 d of age (derived from 16–17-d-old CBA/H embryos or from BALB/c newborn donors) or with a thymus graft from CBA/HT6T6 origin enclosed in a diffusion chamber prepared with filters of a mean pore size of 0.10 μ m as described before¹⁴. Some experimental *nu/nu* mice were grafted at 30 d old with allogeneic C57BL/6 skin and these animals retained the grafted skin throughout the experiment. All animals were observed for 50 d after M-MSV injection.

Table 1 shows the results of such experiments. M-MSV induced 100% local tumours in every experimental group; however, differences in the mean latent periods for local tumour development were observed. While in the *nu/+*, CBA/H or BALB/c controls (experimental groups 1, 6, 7 and 12) the mean latent period ranged from 5.5 to 7.0 d, the latent periods for tumour development in the *nu/nu* groups (groups 2 and 8) were 19.1 and 16.8 respectively, which are significantly prolonged compared with any of the control groups. The same prolonged latent periods for tumour development were observed in the two groups of *nu/nu* mice grafted with allogeneic skin to monitor immune incompetence (groups 3 and 9). Conversely, the *nu/nu* mice grafted with a free thymus graft (groups 4 and 10) behaved as the normal *nu/+* and had short latent periods for tumour development (7.1 and 6.9 d respectively). On the other hand, thymuses enclosed in a diffusion chamber were incapable of shortening the long latent period for tumour development in the *nu/nu* (group 5). A small additional group (group 11) of *nu/nu*-BALB mice injected intraperitoneally at 30 d of age with 100×10^6 cells from 60-d-old *nu/+* BALB thymuses showed

only a slight prolongation of the latent period for tumour appearance when compared with controls.

Within the groups of *nu/+* mice, immune competence of some animals (16 in group 1 and 7 in group 7) was tested with grafts of C57BL/6 allogeneic skin at 30 d of age. Skin graft rejection within 12 d of grafting was observed in every instance (9.9. and 9.2 days respectively) and since such groups did not differ from the non-grafted animals in behaviour towards M-MSV infection, they were not considered as separate groups in Table 1.

Table 1 also shows the total incapacity of *nu/nu* mice to produce regressions of the developing tumours (groups 2, 3, 8 and 9). Conversely, the immunologically normal controls (groups 1, 6, 7 and 12) produced regressions in almost every instance within 10–12 d after tumour appearance. The thymus

suggesting that the target cell for the humoral activity of the thymus in the peripheral lymphoid tissues is a post-thymic cell which is absent in the *nu/nu* mice¹⁴.

The delay in tumour appearance in *nu/nu* may be explained by the inability of these mice to develop the early atypical granuloma which is a common feature of the M-MSV-induced tumours in normal mice²³. Analysis of the cellular composition of the tumours appearing in an additional group of 5 *nu/nu* and 4 *nu/+* (on BALB/c background) supported this interpretation. The cellular composition, studied 7 d after tumour appearance (cells dispersed by 30 min incubation in 0.25% pronase) was 25–30% tumour cells in the *nu/+* versus 43–66% in the *nu/nu*, the remaining cells being lymphocytes, polymorphs and macrophages. The proportion of macrophages within the tumours (determined by ability to ingest latex particles) was

Table 1 Tumour incidence and regression in *nu/nu*, *nu/+*, CBA/H and BALB/c mice after injection of M-MSV

Experimental groups	No. with tumours per total injected	Mean latent period for tumour appearance (d ± s.d.)	No. of tumours regressing	Mean regression time (d ± s.d.)
(1) <i>nu/+</i> (CBA)	39/39	6.5 ± 1.9	39 (100%)	10.8 ± 2.1
(2) <i>nu/nu</i> (CBA)	21/21	19.1 ± 3.7	0	—
(3) <i>nu/nu</i> (CBA-Sk)	10/10	19.6 ± 2.5	0	—
(4) <i>nu/nu</i> (CBA-TG)	12/12	7.1 ± 1.4	11 (92%)	10.6 ± 1.6
(5) <i>nu/nu</i> (CBA-TDC)	10/10	16.9 ± 2.7	0	—
(6) CBA/H	20/20	7.0 ± 1.6	19 (95%)	12.1 ± 1.9
(7) <i>nu/+</i> (BALB)	16/16	5.5 ± 1.2	14 (87%)	10.2 ± 1.8
(8) <i>nu/nu</i> (BALB)	12/12	16.8 ± 2.4	0	—
(9) <i>nu/nu</i> (BALB/Sk)	7/7	17.2 ± 2.1	0	—
(10) <i>nu/nu</i> (BALB-TG)	12/12	6.9 ± 1.5	10 (83%)	10.6 ± 1.5
(11) <i>nu/nu</i> (BALB-Thy)	6/6	10.0 ± 2.1	2 (33%)	13.5 ± (12–15)*
(12) BALB/c	20/20	5.8 ± 1.2	17 (85%)	10.6 ± 1.6

All mice were injected at 50 d of age intramuscularly in the left thigh with 0.1 ml of 1:2 diluted M-MSV containing $5-8 \times 10^6$ focus forming units per ml when tested in secondary BALB/c mouse embryo cell cultures. The original stock of M-MSV was obtained from Electro-Nucleonics Laboratories, Bethesda as M-MSV-infected BALB-3T3 cells. All virus used in the present experiments was prepared as in ref. 15 as 1 g ml^{-1} equivalents from M-MSV tumours produced in BALB/c mice, divided into aliquots and stored at -75°C . The virus used was apparently free of LDH-elevating virus, since infected mice showed normal LDH serum levels, measured as in ref. 13. 'Tumours' indicates development of nodules at the injection site. CBA indicates those *nu/nu* and *nu/+* mice which are in CBA/H background. BALB indicates those *nu/nu* and *nu/+* which are in BALB/c background. Sk indicates allogeneic C57BL/6 skin grafts. TG indicates a thymus graft implanted intraperitoneally (i.p.). TDC indicates a thymus within a diffusion chamber implanted i.p. Thy. indicates the i.p. injection of 100×10^6 thymocytes from adult *nu/+* BALB mice. All these procedures were performed at 30 d of age. All animals were observed for 50 d after M-MSV injection. Mean regression times were calculated excluding those animals that did not show regression. Asterisk indicates actual regression times. By Mann-Whitney non-parametric tests¹⁶ the differences in regression times between non-overlapping populations are significant.

dependency of these regressions was demonstrated by the effect of thymus grafting in the *nu/nu* mice (groups 4 and 10) in which regression was observed in 92 and 83% respectively of the animals. Conversely, thymuses enclosed in a diffusion chamber were ineffective in supporting regression of the developing tumours (group 5). The small group of *nu/nu* BALB injected with dispersed syngeneic thymocytes (group 11) showed regressions in 2 of 6 instances (33%).

These studies show that the capacity to produce regression of M-MSV-induced tumours has a strict thymus dependency. The exact immunological mechanism by which these regressions are produced is still undefined. Both antibodies^{2,5,8,17} and cells⁹ can transfer immunity *in vivo* and both T^{9,18,19} and non-T cells^{10,19} as well as normal lymphocytes in the presence of immune sera²⁰ or immune serum and complement²¹ behave as effector mechanisms in inhibition of tumour growth or cell kill *in vitro*. Whatever the mechanism(s), the thymus is required for the generation of such effector products, whether cells or antibodies. Additional factors may, however, be important since considerable differences in tumour growth and regression were observed between different stocks of M-MSV^{2,22}. The inability to restore the capacity to produce tumour regressions in *nu/nu* mice with thymus enclosed within a diffusion chamber, while free thymus grafts were effective, supports our results

30–41% in the *nu/+* and 17–26% in the *nu/nu*. The results in the *nu/+* mice are comparable with those observed by others in early M-MSV lesions²⁴. When these relative values were expressed as absolute number of cells, the differences increased, since the total number of cells per tumour was higher in the *nu/+* mice (mean values 2×10^8 for *nu/nu* and 6×10^8 for *nu/+* mice), however, variability of the cell yield per tumour precludes interpretation without further study. The present evidence can be interpreted as a consequence of impaired granuloma formation and/or the reduced rate of transformation or reduced rate of proliferation of the transformed cells in the nude mice. These differences in cellular composition were also observed in histological sections and are presently under study. This interpretation is also supported by the long latent period for tumour development observed in mice irradiated with 350 r and infected with low doses of M-MSV¹. The proportion of atypical granulomas compared with sarcomas defined histologically was, however, comparable between normal or thymectomised or irradiated mice²³.

The naturally occurring cytotoxic anti-tumour antibodies described in the serum of nude mice²⁵, may also play some role in our observations. These antibodies are complement-dependent and can lyse various tumour cell lines (including two M-MSV lines) and are not detected in *nu/+* mice at any age,

however, the incidence of such antibodies in the sera of individual mice from our colony, both in the BALB/c and CBA/H background, is relatively low, never above 30% of the animals tested at ages ranging from 50 to 120 d (my unpublished results). Of the 12 nudes (BALB/c) in group 8 (Table 1) that were pretyped for presence or absence of such antibodies, three had detectable titres when tested against EL4 cells and M-MSV transformed Swiss and BALB/c cell lines. The two animals with the longest latent period, 21 and 23 d respectively, had no detectable titres. All the *nu*/+ tested (group 7, Table 1) had no detectable titres. It is apparent that additional work, especially correlations of *in vivo* and *in vitro* reactivity using this model may solve some of these quandaries. Alternative interpretations such as the possible stimulatory role of the incipient immune response on tumour growth²⁶ or the possible absence of T cells with suppressor activity in the nude mouse²⁷, cannot be excluded.

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Erratum

In the article "Two types of resistance to polyene antibiotics in *Candida albicans*" by C. C. HsuChen and D. S. Feingold (*Nature*, **251**, 656; 1974) the following corrections should be made. Page 658, column 1, line 6 should read . . . pore size in cell wall (a molecular sieving effect¹⁶); (3) a temperature . . . Page 659, line 19 should read . . . and dinitrophenol reduce the effect of the polyenes . . .

In the legend to Fig. 1, line 4 should read . . . temperature. Only exponentially growing cells were used in these experiments. Washed cells were suspended . . .

In the legend to Fig. 3, line 2, for 139 read E139A; and the expression in lines 6–8 should have square brackets inserted thus, [(glucose released in the presence of polyene—blank control)]

(total amount of glucose trapped—blank control)] × 100 . . .

In the legend to Fig. 4 the explanation of the symbols does not show clearly the relationship between the neutral lipids and phospholipids. The tabulation below clarifies the situation.

	Neutral lipid of	Phospholipid of
●	E139-A	A1
▲	E139-A	E139-A control
■	E139-A	A3
○	A1	A1 control
▼	A1	E139-A
△	A3	A3 control
□	A3	E139-A

Incorrect symbols were inadvertently used in Table 1 which rendered it meaningless. The correct version is reprinted below.

Table 1 Characterisation of polyene-resistant mutants of *C. albicans*

		Polyene added ($\mu\text{g ml}^{-1}$)	Strain					
			E139-A	A1	A2	A3	A4	A7
Growth in liquid medium*	Blank control	0.0	+++	++	+++	++	+++	++
	Amphotericin B	40.0	—	—	—	—	—	—
		20.0	—	+	—	+	—	—
		10.0	—	++	—	++	—	—
		2.5	—	++	—	++	++	—
		0.5	—	++	+++	++	+++	++
Growth on solid medium†	Nystatin	100.0	—	++	—	++	+++	—
		50.0	—	++	+++	++	+++	++
		25.0	—	++	+++	++	+++	++
		10.0	+	++	+++	++	+++	++
		2.5	+++	++	+++	++	+++	++
	Nystatin + amphotericin B	100.0	—	+	+	+	+	+
	100.0	—	+	—	+	+	—	
	20.0	—	+	—	+	+	—	
Colour reaction‡			Greenish blue	Bright yellow	Purplish blue	Bright yellow	Greyish blue	Greyish blue
Doubling time (min)§			65	85	70	97	90	135

* Log phase cells were adjusted to approximately 5×10^4 colony forming units per ml in fresh growth medium; 2 ml of this dilution was added to tubes containing the given polyenes. Dimethyl sulphoxide was present to 1% in all samples including the blank control. After overnight incubation at 37° C, the turbidity was recorded.

† A single colony of the given strain was streaked out on plate containing the given polyenes, and the growth was checked after 24 h of incubation at 37° C. The nystatin ($4,530 \text{ U ml}^{-1}$) and the amphotericin B ($876 \mu\text{g ml}^{-1}$) were gifts from the Squibb Institute of Medical Research.

‡ Liebermann-Burchard reaction used. Total lipid extract of whole cells were used for assay. Preparations from both log and stationary phase cells gave identical results.

§ Growth was monitored turbidimetrically with a Klett-Summerson photoelectric colorimeter (green filter).

reviews

WHEN Maurice Clavelin's *Essay on the Origins and Formation of Classical Mechanics* first appeared in French in 1968, it was widely recognised as a work of much originality, and nothing published since that first appearance has made very serious inroads into his main conclusions. The *Essay* is a long and intensive study of "a transition from one conceptional framework to another, the replacement of one explanatory ideal with another and an unprecedented fusion of reason and reality", and M. Clavelin argues that to evaluate the scope of the revolution "we must try to view Galileo's work from within". He professes to make no systematic reconstruction of the intellectual and social context in which Galilean science evolved, and yet, overlooking these modest protestations, the reader will have no difficulty in extracting just such materials. The book begins with the Aristotelian doctrine of motion—the intrinsic analysis of local motion, the cosmological frame without which local motion (for Aristotle) was inconceivable, and the descriptive analysis of local motion (the classification of it, the evaluation of its magnitude, and resistive force). By emphasising the coherence of the Aristotelian theory, and the interrelations of the cosmos and local motion, M. Clavelin explains how—far from being the heap of inanities some popular histories would have us believe—it could be questioned and extensively qualified by great minds, without collapsing into a pile of rubble. And if that is important to an understanding of Galileo, then the traditions of the 14th century schools of Oxford and Paris was even more so.

In that connection, Galileo for long misled his commentators by lavishing praise on Archimedes at the expense of Aristotle. Of the fact that Galileo's technical language owed much to mediaeval precursors there is no doubt; but how extensive was his debt to them? Like them, Galileo at first tried to relate the increase in speed of a falling body to its spatial position rather than to time. (After all, according to Aristotle, motion depended on position.) The mediaeval doctrines were an established part of the teaching he had at Pisa between 1583 and 1586. In his *Juvenilia* Galileo reveals himself as in many respects an Aristotelian, and his early *Treatise on the Latitude of Forms* includes many allusions to the Mertonians and Parisians. Simultan-

Focusing on Galileo

J. D. North

The Natural Philosophy of Galileo: Essays of the Origins and Formation of Classical Mechanics. By M. Clavelin. Pp. xiii+498. (MIT Press: Cambridge, Massachusetts, and London, 1974). \$25.00. *Two New Sciences.* Including *Centers of Gravity and Force of Percussion.* By Galileo Galilei. Table introduction and notes by Stillman Drake. Pp. xxxix+323. (The University of Wisconsin Press: Madison, Wisconsin, 1974.) \$14.00 cloth; \$5.00 paper.

eously with these early writings, however, Galileo was reading Euclid, Archimedes (especially on hydrostatics), and Tartaglia (on projectiles). These works convinced him of the need to replace the textual criticism and qualitative dialectic of the philosophers with a direct and mathematical method. The first taste of the great power of the essentially geometrical method for the analysis of physical problems was evident in the early works *De motu* and *Mecaniche*. For the greater *Dialogue and Discourses*, in which the method was exploited far more fully, the world had to wait some decades, and this because from 1602 until his death in 1642 Galileo found a need to link Copernicanism with the mathematical science of the motion of heavy bodies. Into this period, of course, came the telescopic discoveries which so distinguished the Galilean outlook. M. Clavelin reminds us how important was the cosmological component in thought about motion, before Newton's time. Galileo, for instance, was never to abandon the view "that circular motion alone had an actual and real power of indefinite self-conversation". Unlike his precursors, we are told, Galileo was able to treat local motion as a state rather than as a process, and Copernicanism indeed set Galileo the problem of justifying the idea of an orderly world in which motion, not rest, is the normal state of the Earth. His analysis of the Earth's diurnal motion (as in the Second Day of the *Dialogue*) led him to create some of the most significant of his mechanical concepts—such as the idea of an inertial system, the principle of the conservation of uniform motion, and the principle of the composition

of motions. M. Clavelin explains all that beautifully, and at length, steering a middle course between the positivism of Duhem and the Platonism of Koyré. He contrasts the contents of the *Dialogue* with the corresponding parts of the *Discourses*, and at the same time emphasises a "lack of cohesion" in Galileo's mechanical ideas which denied him the opportunity of discussing properly the planetary motions in mechanical terms, and which indeed made impossible the treatment of weight as a force.

Before Professor Drake's work, the last important English translation of the *Discourses* (1638) was prepared by Henry Crew and Alfonso de Salvio in 1914 under the title *Dialogues Concerning Two New Sciences*. Earlier English translations had been published in 1665 and 1730. In the introduction to Drake's new translation, reasons are given for thinking a new version desirable. The changes are not such as to strike the casual reader as very significant, but it is the very fact that they are spelt out at length which gives the new version its value. Take the (Italian) noun *mobile*, for instance, signifying a tangible and heavy moving object near the Earth's surface. This is now rendered by the English 'moveable', in contradistinction to the adjective 'movable'. Crew and De Salvio translated *mobile* to mean 'particle', to which objection is now made on the somewhat curious grounds that "the modern physical particle is essentially devoid of weight". As another example, Professor Drake dislikes the introduction of the mediaeval concept of "mean speed" into the statement of Galileo's basic first theorem on accelerated motion, in place of "one-half the final speed". Whatever the reader's feelings over these subtleties, it is undeniable that the new version must now replace the others. It includes original Galileo works which were published posthumously in the form of a dialogue on the force of percussion (intended for the 1638 edition, but not finished to Galileo's satisfaction), and Galileo's work on centres of gravity. Professor Drake, whose reputation as a Galileo scholar is well known, adds some useful notes, as well as a bibliography of the principal editions and translations of the *Two New Sciences*. The new version, like the original Leyden (Elzevirs) edition, is very attractively designed and printed, and not unduly expensive. □

Management of natural systems

Genetics of Forest Ecosystems. By Klaus Stern and Laurence Roche. Pp. 330. (Chapman and Hall: London; Springer: Berlin and New York, 1974.) £12.25.

IN his preface Roche states that forest ecosystems are a rich source of information on ecological genetics but that published evidence has not yet significantly penetrated the botanical literature. Little exception could be taken to the first part of that statement, and the book, itself a rich source of information, goes some way towards correcting the imbalance mentioned in the latter part. But according to the introductory remarks by Stern the book aims to be more than a catalogue of information on stable and transitory forest ecosystems. Stern assumes that forest ecologists and breeders have been more concerned than agronomists with the inner mechanisms of ecosystems and that only by knowing these mechanisms could the former group hope to improve the economics of forestry. Accepting that this is true and that ecologists and breeders significantly affect forestry practice, then discussion of the state of man's knowledge of these mechanisms, which form an important part of the book, is a valuable contribution to the intelligent management of the world's forest resources.

The main subjects—ecological niches, adaptations, genetic systems, adaptive strategies and forest ecosystems—are approached in a similar manner throughout. First, formal definitions are stated and discussed and that is followed by a consideration of mathematical concepts and their limitations and applicability. Finally, examples are given, mainly from the forest ecosystem, to illustrate and complement the previous discussions. The use of the concepts of systems theory, games strategy and cybernetics will provide difficult reading to the uninitiated. Such difficulties are compounded by the odd misprint, the occasional grammatical peculiarities, and a style of writing which may well be a consequence of translation from the original German.

In a book of this scope it is always possible to find something to criticise but I must say that I thought that the chapter on genetic systems of forest trees species placed undue emphasis on selfing, whereas the reader was referred elsewhere for information on other aspects. And there is much space devoted to Fowler's work on *Pinus resinosa* but his conclusions are reported erroneously in the text. On the other hand, there is much of which I approved, such as the discussion of the

tropical ecosystem, which emphasises and contrasts the ideas of Federov and Ashton.

The final chapter, contributed by Roche, is a review of man's effect on the ecosystem. It is proposed in the introduction that this chapter could well have been placed in a separate book. Had it been placed at the beginning of this book, however, its contents would have encouraged those whose activities affect the forest ecosystem to read the following chapters.

Ian R. Brown

Viral diseases

Slow Virus Diseases. Edited by John Hotchin. Pp. xvii+372. (Karger AG: Basel, London and New York, 1974.) £19.80; \$49.75.

THE title of this book is misleading for the text is concerned mainly with persistent viral infections. 'Slow' infections constitute only a small and unrepresentative group. Seventy pages describe the arena virus group of which LCM virus is the prototype. In contrast, the 'slow' virus diseases are described less adequately. Thirty pages outline reasons for viral persistence, and examples of diseases of man and animals in which persistent virus is considered important in pathogenesis are written about with varying clarity by 27 specialists. All in all, the book is a useful introduction to a developing but not well-defined area of medicine. John T. Stamp

Plants beneath the rising Sun

The Flora and Vegetation of Japan. Edited by M. Numata. Pp. x+294. (Kodansha: Tokyo; Elsevier Scientific: London and New York, 1974.) Dfl80; \$30.80.

SINCE 1950, the Japanese have achieved an international reputation for the originality and importance of their contribution to quantitative studies in plant ecology. The analysis by Monsi and Saeki of the attenuation of solar radiation intercepted by herbaceous vegetation, the application by Hozumi of allometric methods to the estimation of growth of forest stands, and the studies by Kira and his associates of intraspecific competition are all examples of work which has attracted world-wide attention. Recognition of the significance of this work has been facilitated by publication in English and German. In contrast, most of the literature on the vegetation of Japan

has been published in Japanese so that it remains relatively inaccessible. Now a concise guide is available in English and it provides an excellent account of one of the most intensively studied and interesting vegetational regions of the world.

Modern Japan extends from the northern coast of Hokkaido (latitude 46° N) to the southernmost of the Ryukyu islands which is almost on the Tropic of Cancer; a distance of over 3,000 km along the great archipelago which stretches from Kamchatka and the Kuril Islands in the north to Formosa (Taiwan) and the Philippines in the south. During the climatic vicissitudes of the Pleistocene, the continuity of the archipelago seems to have allowed much of the Eocene flora to survive by oscillating from north to south and back. Consequently, the flora of Japan is extraordinarily rich, with almost 4,000 vascular plants, including many endemics and relict species of the Arcto-Tertiary flora, and a great wealth of woody species.

Small areas of sub-tropical vegetation occur on the Ryukyu islands but the original vegetation on the main islands ranges from warm temperate forests, comprising largely evergreen broad-leaved species in Kyushu, Shikoku and southern Honshu, to cool temperate forests, characterised by beech (*Fagus crenata*) and representatives of several familiar temperate genera such as *Acer* and *Prunus*, in northern Honshu. Subarctic coniferous forests of *Abies sachalinensis* and *Picea* spp. are present on the mountains of Honshu and descend to sea-level in Hokkaido. This latitudinal zonation is accentuated by the warm Tsushima and Kuro-shio currents which originate in the tropics and have a large influence on the climate of the southern islands but which fail to reach Hokkaido.

Much of the land which would naturally carry forest has been modified greatly and is now occupied by grassland or vegetation dominated by *Pteridium aquilinum*. There is a wide variety of coastal vegetation and extensive regions of subalpine and alpine vegetation, in which a majority of the genera are those important in Europe. The final section is devoted to an account of the vegetation of active volcanoes and includes some remarkable illustrations of recent lava flows and pumice deposits now covered by forests.

The book provides a clear and fascinating account of Japanese vegetation and of the many geographical and ecological problems presented by its complexity and its considerable modification by man. It is well written, well balanced in its presentation and has numerous illustrations which are uniformly of high quality.

C. D. Pigott